

Research Article

1,25-Dihydroxy Vitamin D₃ Facilitates the M2 Polarization and β -Amyloid Uptake by Human Microglia in a TREM2-Dependent Manner

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by dementia as the primary clinical symptom. The production and accumulation of aggregated β -amyloid ($A\beta$) in patient brain tissues is one of the hallmarks of AD pathogenesis. Microglia, brain-resident macrophages, produce inflammatory cytokines in response to $A\beta$ oligomers or fibrils exacerbating $A\beta$ pathology in AD. HMO6 cells were treated with $A\beta_{42}$ in the presence or absence of 1,25-dihydroxy vitamin D₃ ($1,25(\text{OH})_2\text{D}_3$) to determine its potential immunomodulatory effects, and the expression of pro-/anti-inflammatory cytokines, M1/M2-associated markers, Toll-like receptors (TLRs), and triggering receptor expressed on myeloid cells 2 (TREM2) was examined. $1,25(\text{OH})_2\text{D}_3$ was found to suppress $A\beta$ -induced expression of proinflammatory cytokines (TNF- α , IL-1 β , and IL-6), M1 markers (CD86 and iNOS), and TLR2/4, whilst increasing the expression of anti-inflammatory cytokines (IL-4, IL-10, and CCL17) and M2 markers (CD206 and Arg-1). Furthermore, $1,25(\text{OH})_2\text{D}_3$ promoted TREM2 expression and $A\beta$ uptake by HMO6 cells, and the enhancement of $A\beta$ uptake and M2 polarization was revealed to be TREM2-dependent. The findings of this study suggest that $1,25(\text{OH})_2\text{D}_3$ facilitates M2 polarization and $A\beta$ uptake in a TREM2-dependent manner.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that accounts for 60-80% of dementia cases worldwide and is becoming an increasingly severe socioeconomic problem [1]. The major causes of the AD are the extracellular accumulation of beta-amyloid ($A\beta$) plaques and the intracellular accumulation of neurofibrillary tangles containing hyperphosphorylated tau proteins [2]. The accumulation of $A\beta$ peptide is associated with proteolytic cleavage of the membrane $A\beta$ precursor protein (APP), which is cleaved by β - and γ -secretases to yield a 37-49 amino acid residue peptide, $A\beta$ [3]. Among the several APP cleavage products that contribute to AD, the 40- and 42-amino acid forms ($A\beta_{40}$ and $A\beta_{42}$, respectively) are the main final $A\beta$ products [4]. $A\beta_{42}$ is the more toxic form, which is attributable to the additional two amino acids that lead to misfolding and subsequent self-aggregation [5]. The accumulation of the more insoluble $A\beta_{42}$ aggregates

functions as a trigger for AD pathogenesis [6]. Aggregated extracellular $A\beta$ may stimulate the activation of microglia, which are brain-resident macrophages that play a pivotal role in AD-associated neuroinflammation [7]. Activated microglia produce proinflammatory cytokines that are typically detected surrounding extracellular $A\beta$ plaques in the brains of AD patients [8], and overactivation of these microglia promotes the development of inflammatory injuries and exacerbates the AD-associated pathology [9]. Thus, microglia-mediated inflammation is considered a promising therapeutic target for the treatment of AD.

TREM2 (triggering receptor expressed on myeloid cells 2) is a cell surface receptor expressed by microglia that plays an important role in the central nervous system (CNS) inflammation [10], and mutations in the TREM2 gene have been linked to an increased risk of developing AD [11, 12]. Ectodomain of TREM2 binds to a variety of ligands, including bacterial polysaccharides, lipoproteins, phospholipids, and DNA [13].

When such ligands bind to TREM2, intracellular signaling pathways are activated, which contribute to modulating cellular functions such as increased cell survival [14, 15], phagocytosis [14], cell adhesion and migration, and cytokine secretion [14]. Microglial TREM2 also detects extracellular A β , and its structure and function have been identified to influence the clearance and aggregation of A β [16–18].

The active form of vitamin D, 1,25-dihydroxy vitamin D₃ (1,25(OH)₂D₃), is essential for calcium absorption and bone mineralization and is involved in a variety of biological processes, including cell growth and differentiation and immune response [19]. 1,25(OH)₂D₃ regulates target gene expression by binding to a nuclear hormone receptor, the vitamin D receptor (VDR) [20]. Emerging evidence suggests that vitamin D is involved in the early development of animal brains [21] and that maternal vitamin D deficiency during pregnancy is correlated with cognitive development disorders of offspring [22]. Importantly, patients with neurodegenerative diseases, including AD, have lower vitamin D serum levels [23, 24], and recent clinical studies have indicated the potentially beneficial effects of vitamin D supplements in the prevention of cognitive decline in AD patients [25, 26].

In this study, we treated human microglial cells (HMO6) with A β to mimic in vivo microglial activation and neuroinflammation in response to A β accumulation, which are the main pathological features of AD, and examined the potential immunomodulatory effects of 1,25(OH)₂D₃ in vitro.

2. Materials and Methods

2.1. Chemicals and Antibodies. Synthetic A β _{1–42} peptides (DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA) were purchased from LifeTein (Beijing, China); 1,25(OH)₂D₃ was purchased from Sigma-Aldrich (Sigma-Aldrich, Saint Louis, MO, USA); antibodies for TLR4, CD86, CD206, iNOS, and Arg-1 were obtained from Santa Cruz Biotechnology (Dallas, TX, USA); antibodies for TREM2 (B-3) and A β were purchased from BioLegend (San Diego, CA, USA); anti-TLR2 antibody was purchased from Novus Biologicals (Littleton, CO, USA); and TREM2 antibody (237920; R&D Systems, Minneapolis, MN, USA) was selected as blocking antibody according to a previous study [27].

2.2. Cell Culture and the Preparation of A β and 1,25(OH)₂D₃. An established human microglial cell line [28], HMO6 (accession number CVCL_5G94), was used for this study. Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% antibiotic-antimycotic (Invitrogen Corp.), in a 5% CO₂ at 37°C.

The A β _{1–42} peptides and 1,25(OH)₂D₃ were dissolved in 5 mM dimethyl sulfoxide (DMSO) to prepare stock solutions and stored at -20°C. Before use, aliquots of these stock solutions were diluted to the requisite concentrations using DMEM containing 1% antibiotic-antimycotic.

2.3. RNA Preparation and Real-Time Quantitative PCR. Total RNA was extracted using an easy-BLUE™ Total

RNA Extraction Kit (iNtRON Biotechnology, Inc., Seongnam, Korea) according to the manufacturer's instructions. The concentration of the extracted RNA was measured using a MaestroNano MicroVolume Spectrophotometer (Maestrogen, Las Vegas, NV, USA). Aliquots (2 μ g) of the purified RNA were used for the synthesis of cDNA using a HyperScript™ 2 \times RT Master Mix (GeneAll Biotechnology, Seoul, Korea). Quantitative real-time PCR was performed on a Rotor-Gene system (Qiagen) using a QuantiSpeed SYBR NO-ROX kit (PhileKorea, Seoul, Korea). Amplifications were performed using the primer sets listed in Table 1. Sample normalization was performed using the human *GAPDH* gene as an endogenous control. For each sample, the relative abundance of the target mRNA was calculated from the $-\Delta$ cycle threshold (Δ C_t) values of the target and endogenous *GAPDH* reference genes using the $2^{-\Delta\Delta C_t}$ method.

2.4. Flow Cytometry. To examine expression levels of the cell surface proteins TLR2, TLR4, CD86, CD206, and TREM2, cells were collected, washed with DPBS 2 times, incubated with primary antibody at 4°C for 30 min, and followed by phycoerythrin- (PE-) conjugated secondary antibodies at 4°C for 30 min. To determine intracellular levels of iNOS and Arg1, cells were treated with 4% formaldehyde and 1% Triton at room temperature for 20 min. Then, cells were stained with primary antibody at 4°C for 30 min, followed by staining with PE-conjugated secondary antibodies at 4°C for 30 min. Cells were resuspended in phosphate-buffered saline (PBS) and analyzed on a Cytomics FC500 MLP (Beckman Coulter Inc., Fullerton, CA, USA).

2.5. Enzyme-Linked Immunosorbent Assay (ELISA) for A β Measurements. The culture supernatant was collected and added to the 96-well culture plate overnight at 4°C. To prevent nonspecific binding of antigens and antibodies to the well, bovine serum albumin (BSA) 5% was used as a blocking agent. Quantification of soluble A β _{1–42} was performed using anti-A β primary antibody and followed by incubation with m-IgG κ BP-HRP secondary antibody at RT. Absorbance of ELISA test was detected at 450 nm using microplate reader.

2.6. Statistical Analysis. The experiments were conducted at least three times, and all data are independently showed as mean \pm standard deviation (SD). Significant differences among groups were analyzed by one-way analysis of variance (ANOVA) followed by post hoc test using SPSS 12.0 for Windows. Differences were considered statistically significant at *p* value less than 0.05. *T*-test was applied to determine significance among the groups of A β 42 uptake.

3. Results

3.1. 1,25(OH)₂D₃ Downregulates A β -Induced Proinflammatory Cytokines and Upregulates Anti-Inflammatory Cytokines. Given that A β is an aggregation-prone peptide and that monomeric A β can be converted to not only stable oligomeric forms but also short fibrillar A β under physiologic solution conditions at higher A β concentrations [29, 30], we used monomeric A β 42 to stimulate microglia in this study. Initially, we examined the effects of A β 42 on the expression of selected

TABLE 1: qRT-PCR primers used in this study.

Gene name	Forward primer (5'-3')	Reverse primer (5'-3')
GAPDH	ACAGCCTCAAGATCA TCAGCAAT	AGGAAATGAGCTTGACAAAAGTGG
IL-1 β	GGGATAACGAGGCTTA TGTGC	AGGTGGAGAGCTTTCAGTTCA
TNF- α	CAGAGGGCCTGTACCTCATC	GGAAGACCCCTCCCAGATAG
IL-6	GACCCAACCACAAATGCCAG	GAGTTGTCATGTCCTGCAGC
IL-4	CCGTAACAGACATCTTTGCTGCC	GAGTGTCTTCTCATGGTGGCT
IL-10	TCTCCGAGATGCGTTCAGCAGA	TCAGA CAAGGCTTGGCAACCCA
CCL17	ACCCAACAACAAGAGAGTGA	GAGGGCCCAGGTAGTCCC

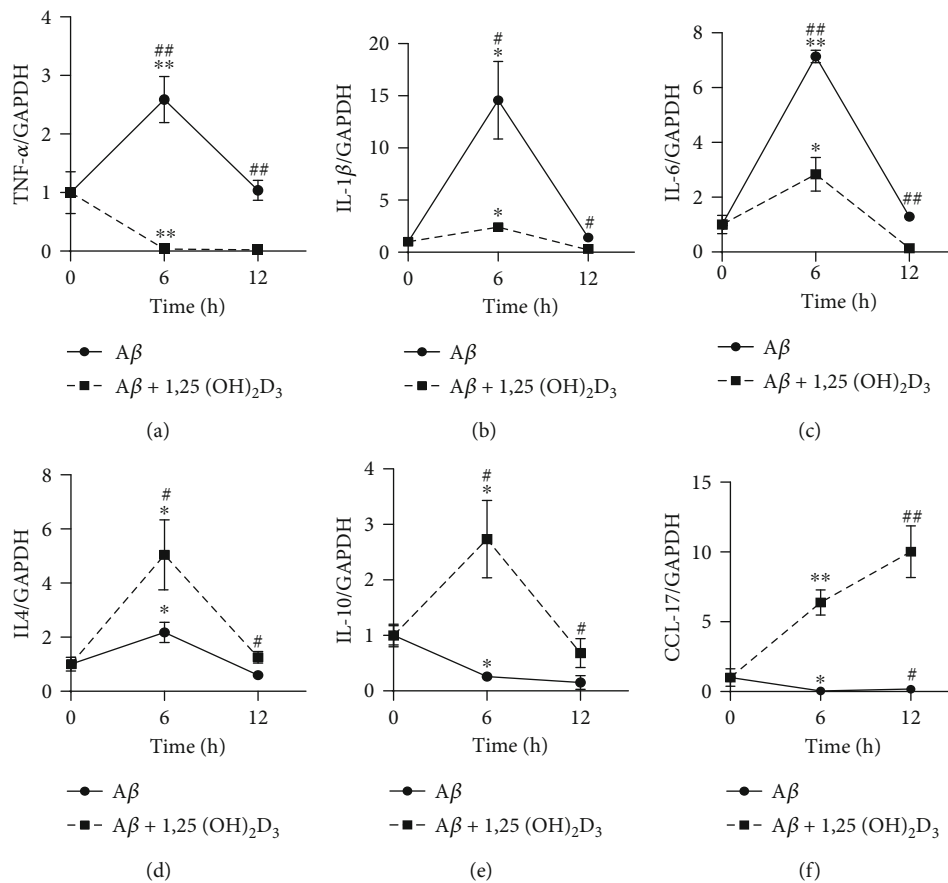


FIGURE 1: 1,25(OH)₂D₃ downregulates A β -induced M1 cytokine expression, whilst upregulating M2 cytokine expression in HMO6 cells. Cells were incubated in DMEM exposed to 100 nM A β ₁₋₄₂ and treated with or without 1 μ M of 1,25(OH)₂D₃ for 6 h or 12 h. mRNA was extracted, and expression of proinflammatory cytokines TNF- α (a), IL-1 β (b), and IL-6 (c) or anti-inflammatory cytokines IL-4 (d), IL-10 (e), and CCL17 (f) was measured by quantitative real-time PCR. * p < 0.05 and ** p < 0.005 vs. 0 h control group; # p < 0.05 and ## p < 0.005 vs. A β ₁₋₄₂-treated group.

cytokines in the HMO6 human microglial cell line. qRT-PCR analysis revealed that mRNA expression of the proinflammatory cytokines TNF- α , IL-1 β , and IL-6 was significantly increased at 6 h after treatment with 100 nM A β 42, but thereafter, expression declined to pretreatment levels at 12 h. However, the expression of these cytokines was markedly inhibited when the HMO6 cells were treated with both 100 nM A β 42 and 1 μ M 1,25(OH)₂D₃ (Figures 1(a)–1(c)). Furthermore, we detected a significant reduction in the expression of the M2 cytokines IL-10 and CCL17 in A β 42-treated cells, whereas that of IL-4 was increased. However, in response to the com-

bined treatment with 100 nM A β 42 and 1 μ M 1,25(OH)₂D₃, there were significant increases in the expression of these cytokines (Figures 1(d)–1(f)). These findings accordingly indicate that 1,25(OH)₂D₃ can reverse the proinflammatory cytokine expression induced by A β 42 and enhance the expression of anti-inflammatory cytokines.

3.2. 1,25(OH)₂D₃ Reverses the A β -Induced Upregulation of TLR4 and TLR2 Expressions in HMO6 Cells. Toll-like receptors (TLRs) are implicated in a range of neurological disorders, including AD, and are accordingly emerging as

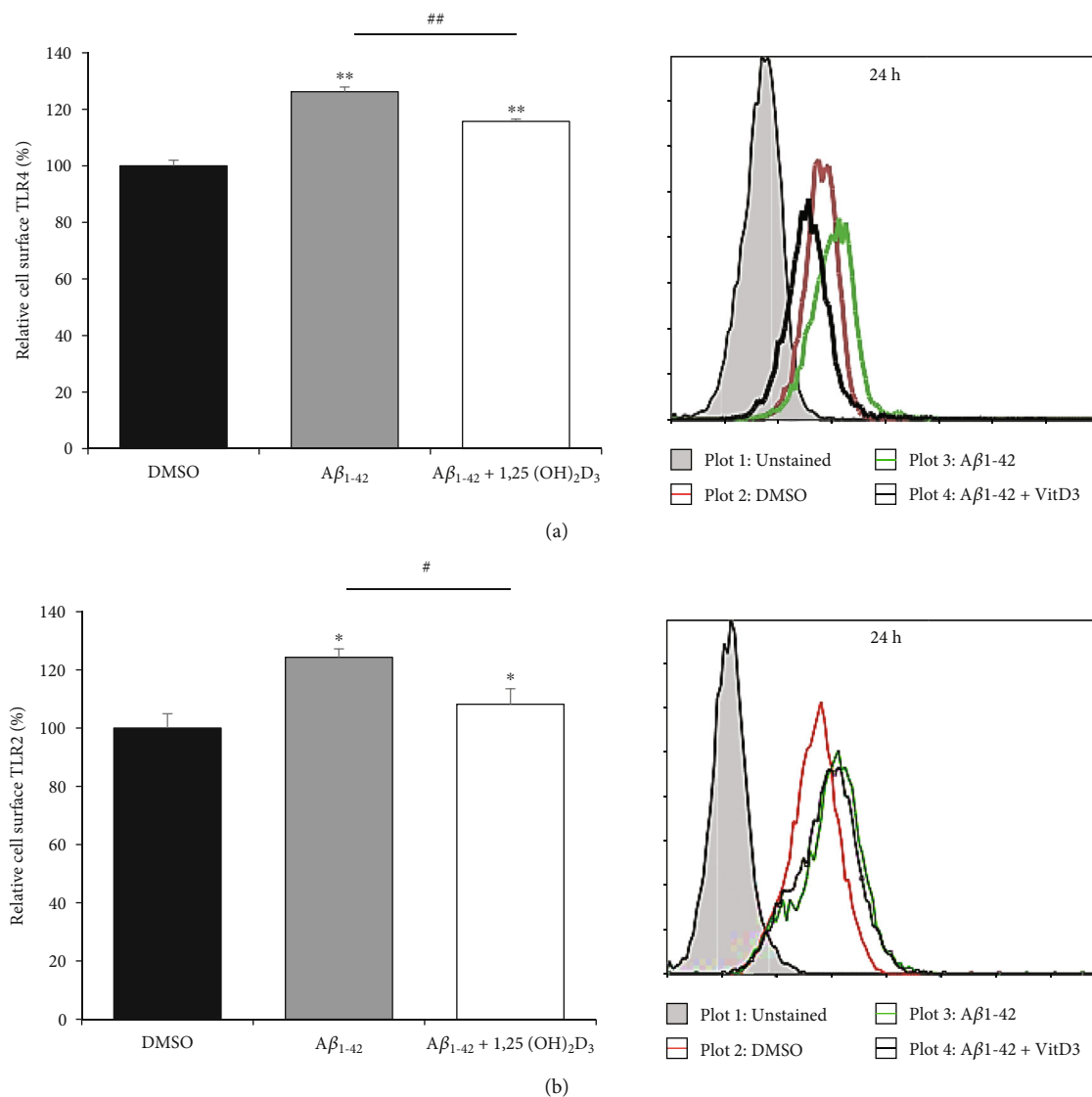


FIGURE 2: 1,25(OH)₂D₃ downregulates the Aβ₄₂-induced expression of TLR4 and TLR2 in HMO6 cells. Cells were treated with or without 1 μM 1,25(OH)₂D₃ in the presence of 100 nM Aβ₄₂ at 37°C for 24 h. Cell surface levels of TLR4 (a) and TLR2 (b) were measured by flow cytometry. Bar graphs indicate the relative expression ± SD (left panels). Right panels designate representative histograms. **p* < 0.05 and ***p* < 0.0005 vs. the DMSO control. #*p* < 0.05 and ##*p* < 0.0005.

promising therapeutic targets for AD treatment [31]. Among the 10 human TLRs identified, TLR4 and TLR2 are reported to function as microglial Aβ receptors [32], which can induce proinflammatory cytokine production via the transcription factor activation of nuclear factor- (NF-) κB in response to Aβ treatment [33]. We thus examined the surface expression of TLR4 and TLR2 on Aβ₄₂-treated HMO6 cells. As shown in Figure 2, there was a significant enhancement of in the expression of these two receptors in Aβ₄₂-treated cells, which was markedly reversed following treatment with 1 μM 1,25(OH)₂D₃.

3.3. 1,25(OH)₂D₃ Downregulates Aβ-Induced M1 Marker Expression in HMO6 Cells, Whilst Upregulating M2 Marker Expression. Similar to macrophages, microglia can be classified into two distinct phenotypes based on their activation

state, namely, M1 microglia, which produce proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6 [34], and contribute to inducing neuronal damage [35], and M2 microglia, which release anti-inflammatory cytokines, such as IL-4 and IL-10, and the Th2 chemokine CCL17 [36] and play role in neuroprotection [37]. In the present study, we examined the effects of Aβ₄₂ treatment on the expression of M1 and M2 markers in HMO6 cells. In Aβ₄₂-treated cells, we detected a significant increase in the expression of the M1 markers CD86 and iNOS, which was reversed following treatment with 1,25(OH)₂D₃ (Figures 3(a) and 3(b)). Expression of the M2 marker arginase I (Arg-1), which metabolically dampens T-cell responses by causing arginine deprivation [38], in Aβ₄₂-treated cells was significantly reduced, whereas that of CD206 remained unchanged. However, in cells cotreated with Aβ₄₂ and 1,25(OH)₂D₃, we

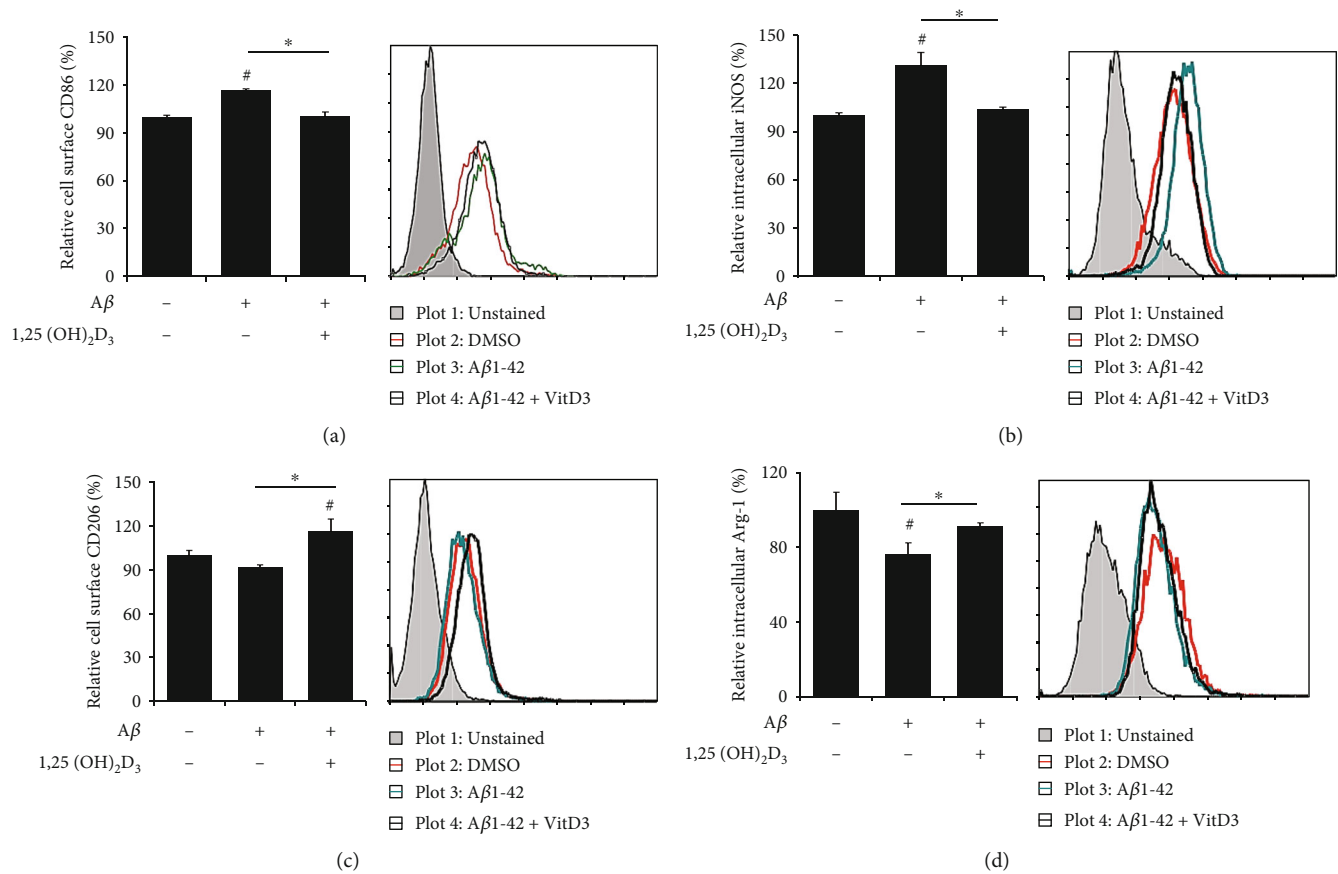
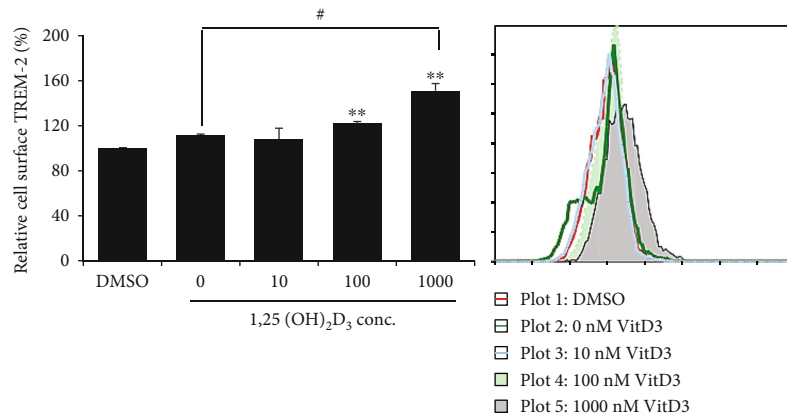


FIGURE 3: 1,25(OH)₂D₃ downregulates Aβ42-induced M1 marker expression in HMO6 cells, whilst upregulating M2 marker expression. Cells were treated with or without 1 μM 1,25(OH)₂D₃ in the presence of 100 nM Aβ42 for 12 h, and protein expressions of M1 markers CD86 (a) and iNOS (b) and M2 markers CD206 (c) and Arg-1 (d) were measured by flow cytometry. Bar graphs indicate the relative expression ± SD (left panels). Right panels designate representative histograms. **p* < 0.05 vs. the DMSO control; #*p* < 0.05 vs. the Aβ42-treated group.

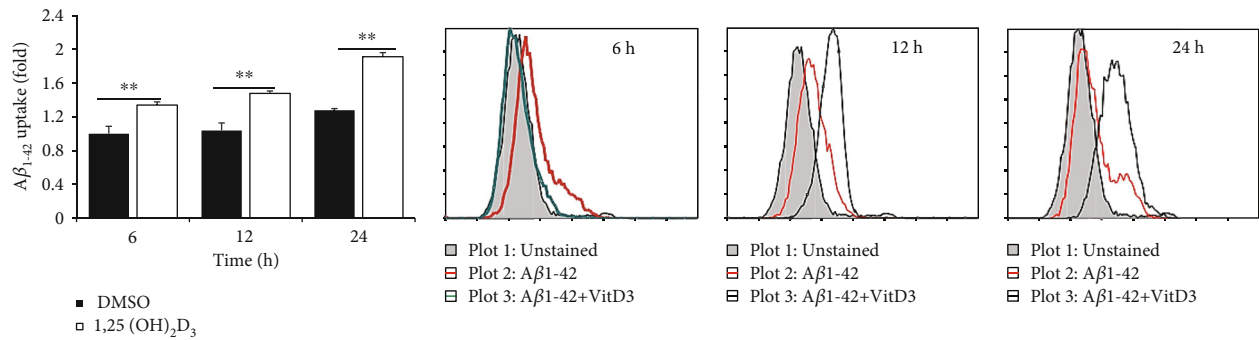
detected the markedly enhanced expression of both these M2 markers (Figures 3(c) and 3(d)). These findings thus provide evidence to indicate that Aβ42 treatment leads to M1 polarization in HMO6 cells and that 1,25(OH)₂D₃ skews the Aβ42-induced M1 phenotype toward an M2 phenotype in these cells.

3.4. 1,25(OH)₂D₃ Enhances Surface TREM2 Expression and Aβ42 Uptake by HMO6 Cells. As a cell surface receptor, TREM2 has been established to interact with Aβ and plays a central role in AD pathogenesis, and thus, we examined the effects of different concentrations of 1,25(OH)₂D₃ on the expression of TREM2 in HMO6 cells treated with 100 nM Aβ42. We accordingly found that when applied at concentrations of 100 and 1000 nM, 1,25(OH)₂D₃ promoted the enhanced expression of cell surface TREM2 in HMO6 cells treated with 100 nM Aβ42 (Figure 4(a)). To determine the capacity of human microglial cells to take up exogenous Aβ42 peptides, HMO6 cells were treated with 100 nM Aβ42 in the presence or absence of 1 μM 1,25(OH)₂D₃ for different periods (6-24 h). Uptake of Aβ42 by HMO6 cells was determined by measuring the intracellular levels of Aβ42 by flow cytometry, and nonuptake of Aβ42 was accessed

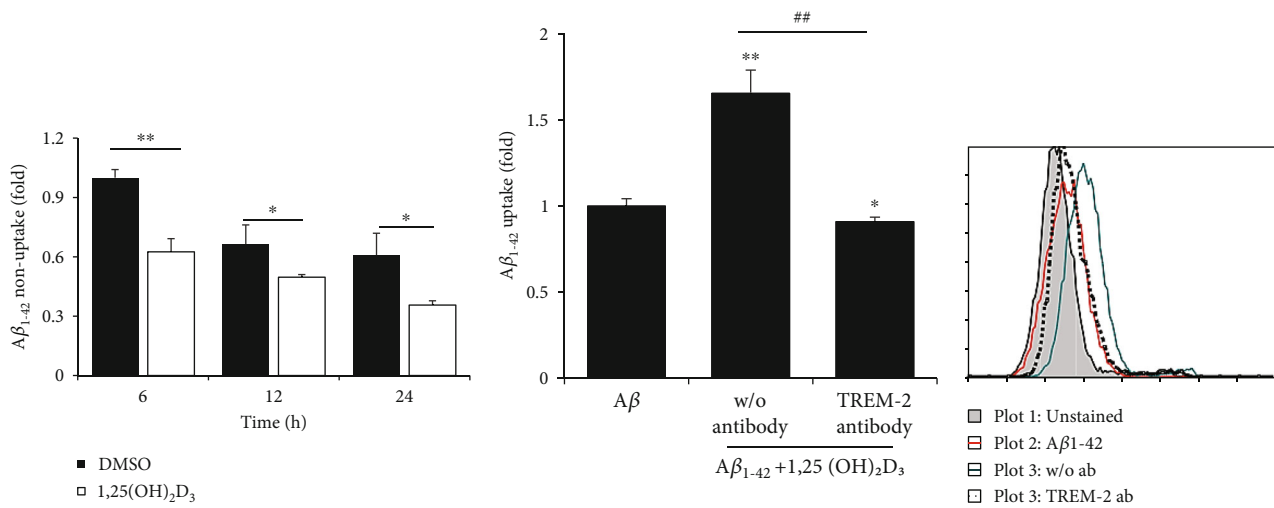
by measuring levels of Aβ42 in culture supernatants based on ELISA. The results revealed that at all assessed time points, the levels of Aβ42 uptake by HMO6 cells cultured in the presence of 1,25(OH)₂D₃ were higher than those in the absence of 1,25(OH)₂D₃ (Figure 4(b)). In line with expectation, nonuptake levels of Aβ42 in the culture supernatants of 1,25(OH)₂D₃-treated cells were significantly lower than those in the supernatants of nontreated cells (Figure 4(c)). These findings thus indicate that 1,25(OH)₂D₃ facilitates the uptake of exogenous Aβ42 by HMO6 cells. The phagocytosis of Aβ by microglial cells has been reported to be dependent on TREM2 [39], and thus, to determine the potential involvement of cell surface TREM2 in the 1,25(OH)₂D₃-enhanced uptake of exogenous Aβ42 by HMO6 cells, we used a TREM2-blocking antibody to block these receptors. In HMO6 cells pretreated with anti-TREM2 antibodies before treatment with Aβ42, we detected significantly lower levels of intracellular Aβ42 than in nontreated cells and even in DMSO-treated cells (Figure 4(d)). Correspondingly, thus nonuptake levels of Aβ42 in the culture supernatants of anti-TREM2 antibody-treated cells were significantly higher than those of nontreated cells and DMSO-treated cells (Figure 4(e)). These observations



(a)



(b)



(c)

(d)

FIGURE 4: Continued.

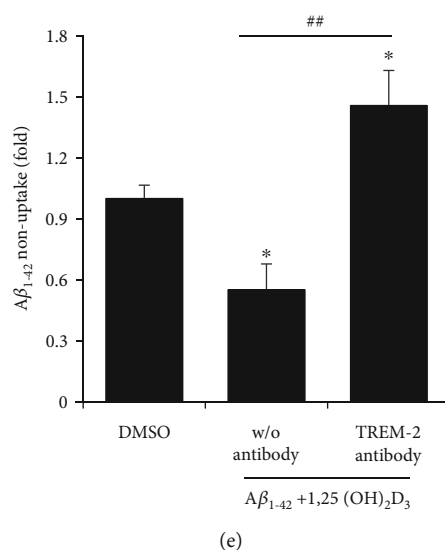


FIGURE 4: 1,25(OH)₂D₃ enhances surface TREM2 expression and Aβ₄₂ uptake by HMO6 cells. (a) Cells were treated with different concentrations of 1,25(OH)₂D₃ in the presence of 100 nM Aβ₄₂ for 12 h, and cell surface expression of TREM2 was measured by flow cytometry. (b) Cells were treated with 100 nM Aβ₄₂ in the presence or absence of 1 μM 1,25(OH)₂D₃ at 37°C for different periods, and the levels of intracellular Aβ were measured by flow cytometry using anti-Aβ antibodies. (c) The culture supernatants were collected, and levels of Aβ were measured by ELISA. (d) Cells were treated with or without TREM2-blocking antibodies for 2 h and then treated with 100 nM Aβ₄₂ in the presence or absence of 1 μM 1,25(OH)₂D₃ at 37°C for 24 h, after which, the levels of intracellular Aβ were measured by flow cytometry. Bar graphs indicate the relative expression ± SD (left panels). Right panels designate representative histograms. (e) Culture supernatants were collected, and levels of Aβ were measured by ELISA. **p* < 0.05 and ***p* < 0.005 vs. the DMSO control. ##*p* < 0.005.

accordingly provide convincing evidence to indicate that cell surface TREM2 is involved in the 1,25(OH)₂D₃-enhanced uptake of exogenous Aβ₄₂ by HMO6 cells.

3.5. 1,25(OH)₂D₃-Induced M2 Polarization Is Dependent on Cell Surface TREM2. Given that TREM2 has been proposed to play a vital role in the anti-inflammatory responses of microglia in AD [40], we examined the expression of M1/M2 markers and pro-/anti-inflammatory cytokines in HMO6 cells following stimulation with Aβ₄₂ in the presence or absence of either 1,25(OH)₂D₃ or anti-TREM2-blocking antibody. Upon exposure to Aβ₄₂, we detected a significant enhancement of M1 marker CD86 expression in HMO6 cells, whereas that of the M2 marker CD206 was reduced, and we found that both these responses could be reversed in the presence of 1,25(OH)₂D₃ (Figures 5(a) and 5(b)). However, the reduced expression of CD86 and enhanced expression of CD206 in 1,25(OH)₂D₃-treated cells were reversed in cells exposed to anti-TREM2-blocking antibodies. Similar to the expression patterns of M1/M2 markers detected upon challenge with Aβ₄₂, the mRNA expression of IL-1β was significantly enhanced, whereas IL-4 was reduced in HMO6 cells, and these responses were reversed in the presence of 1,25(OH)₂D₃ (Figures 5(c) and 5(d)). Again, these 1,25(OH)₂D₃-mediated effects were reversed in the presence of anti-TREM2-blocking antibodies. These findings thus indicate that the cell surface receptor TREM2 may play a role in the anti-inflammatory responses of human microglial cells by facilitating M2 polarization.

4. Discussion

In this study, we demonstrated that in Aβ₄₂-treated human microglial cells, the active form of vitamin D, 1,25(OH)₂D₃, promotes the downregulated expression of the proinflammatory cytokines TNF-α, IL-1β, and IL-6 and induces the upregulated expression of the anti-inflammatory cytokines IL-4, IL-10, and CCL17. In the CNS, microglia can recognize extracellular Aβ oligomers and fibrils and are thereby activated with the associated release of proinflammatory cytokines [41]. The sustained production of these cytokines from activated microglia can exacerbate the AD process [42]. In addition to their direct adverse effect on neighboring neurons [43, 44], the proinflammatory cytokines secreted by activated microglia can activate astrocytes that contribute to promoting neuronal loss in AD [45]. In this regard, the downregulation of proinflammatory cytokines and upregulation of anti-inflammatory cytokines induced by 1,25(OH)₂D₃ in Aβ₄₂-treated HMO6 cells indicate that 1,25(OH)₂D₃ may have applicability in the treatment of AD. Interestingly, IL-4 was increased at 6 h after Aβ treatment in the absence of 1,25(OH)₂D₃. Mechanisms underlying the induction of IL-4 expression in microglia after Aβ treatment are currently unknown. Possible explanation for this induction is that IL-4 may be involved in counteracting the inflammatory response of microglia to Aβ or enhancing the phagocytic activity of microglia to promote the uptake and clearance of Aβ [46]. The application of 1,25(OH)₂D₃ has also been demonstrated to reduce the expression of TLR2 and TLR4, which are cell surface receptors for Aβ,

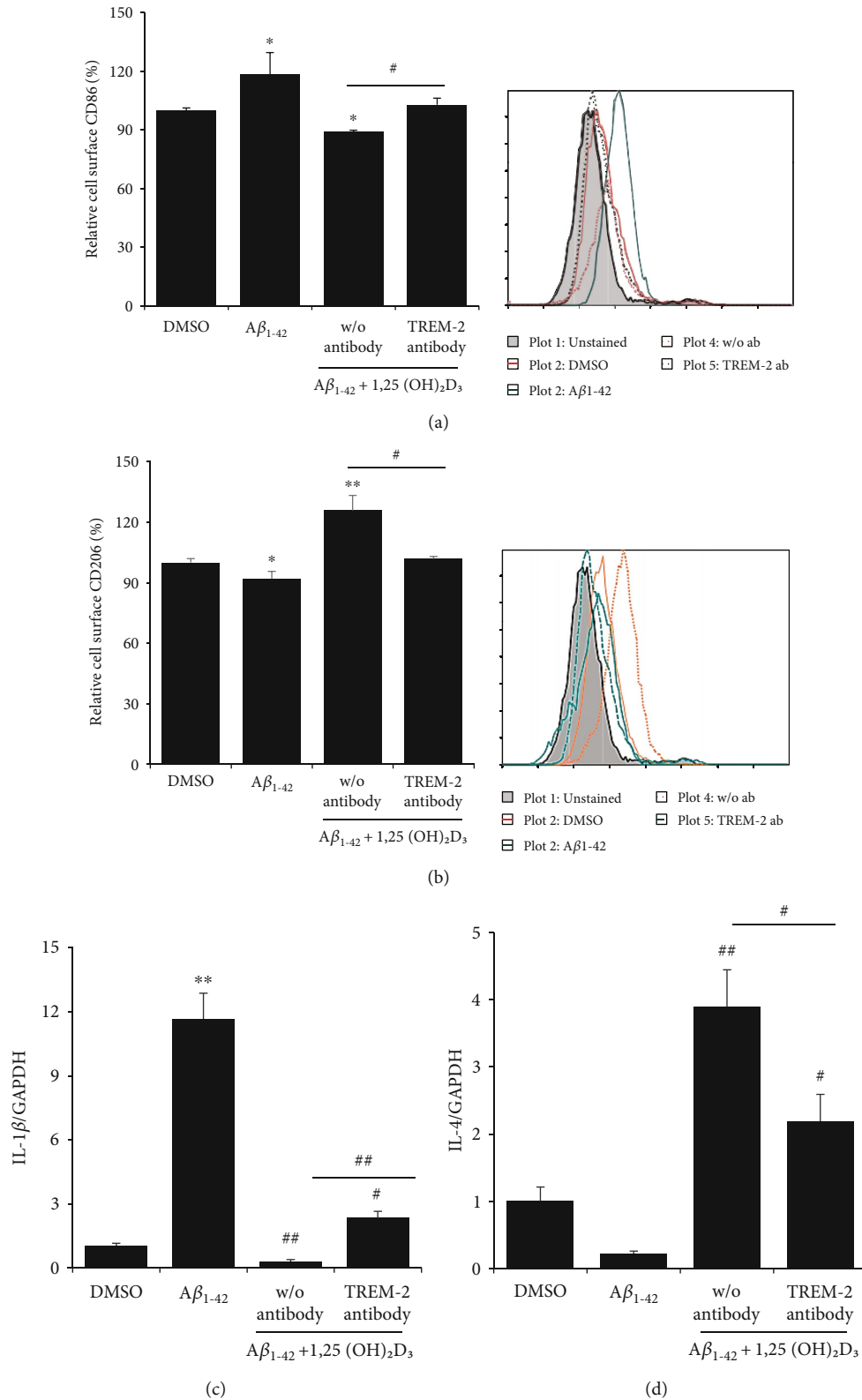


FIGURE 5: 1,25(OH) $_2$ D $_3$ -induced M2 polarization of microglia is dependent on cell surface TREM2. HMO6 cells were pretreated with or without TREM2-blocking antibody for 2 h and then treated with or without 1 μ M 1,25(OH) $_2$ D $_3$ in the presence of 100 nM A β 42 at 37°C for 6 h (for mRNA expression) or 12 h (surface protein expression). Levels of cell surface CD86 (a) and CD206 (b) were measured by flow cytometry. Bar graphs indicate the relative expression \pm SD (left panels). Right panels designate representative histograms. Levels of IL-1 β (c) and IL-4 (d) mRNA expression were analyzed based on quantitative real-time PCR. * p < 0.05 and ** p < 0.005 vs. the DMSO control; # p < 0.05 and ## p < 0.005 vs. the A β 42-treated group.

the activation of which in turn induces microglial activation and neuroinflammation in AD [32]. The A β -induced activation of TLR2 and TLR4 promotes intracellular signaling leading to the production of proinflammatory cytokines. Consequently, the observed reduction in the expression of such proinflammatory cytokines in A β 42-treated HMO6 cells in the presence of 1,25(OH) $_2$ D $_3$ can probably be ascribed to the 1,25(OH) $_2$ D $_3$ -induced downregulation of TLR2 and TLR4 expressions.

Given that A β aggregates induce M1 microglial polarization [45] and that, in turn, the M1 inflammatory response led to the formation of A β aggregates [45, 47], it is important to facilitate either M2 polarization or A β clearance to suppress the progression of AD. In this regard, it is noteworthy that in A β 42-treated HMO6 cells, 1,25(OH) $_2$ D $_3$ downregulates M1 marker expression, whilst upregulating M2 marker expression, thereby indicating that 1,25(OH) $_2$ D $_3$ has the effect of inducing M2 polarization. In this context, it has recently been suggested that promoting a phenotypic shift from M1 to M2 microglia may have therapeutic potential for the treatment of AD [48, 49], and accordingly, the 1,25(OH) $_2$ D $_3$ -induced polarization of a microglial phenotype from M1 to M2 could be considered as a promising therapeutic option.

Microglia play a central role in the clearance of A β aggregates via phagocytosis or receptor-mediated endocytosis, which are mediated by several types of cell surface receptors, including scavenger receptors, receptors for advanced glycation end products (RAGE), lipoprotein receptor-related proteins (LRPs), and TREM2 [50], among which TREM2 has been the most extensively studied. On the surface of microglial cells, TREM2 binds directly to A β oligomers with high affinity, and either the loss [51] or alteration [17] of TREM2 has been found to impair A β clearance by microglia. Consequently, it would appear that the maintenance of sufficient levels of TREM2 expression and function is important for retarding the progression of AD [52]. Our findings in this study revealed that 1,25(OH) $_2$ D $_3$ enhances surface TREM2 expression and extracellular A β 42 uptake by microglia and that the 1,25(OH) $_2$ D $_3$ -induced increase in microglial A β 42 uptake is almost completely reversed in the presence of a TREM2-blocking antibody. These findings indicate that 1,25(OH) $_2$ D $_3$ facilitates microglial A β 42 uptake by upregulating the expression of cell surface TREM2. Consistent with this interpretation, the findings of a recent study have indicated that an agonistic TREM2 antibody, which can induce intracellular signaling, enhances the phagocytosis of oligomeric A β by microglia in vitro and improves cognitive function by attenuating chronic inflammatory responses in a murine model of AD [53, 54].

It is generally accepted that vitamin D plays an essential role in maintaining cognitive function in old age [55] and patients receiving hemodialysis [56], and the findings of several recent meta-analyses have indicated that lower serum levels of vitamin D are associated with a heightened risk of AD [57–60]. Moreover, human and animal studies have indicated that vitamin D contributes a reduction in A β deposition in the brain [61, 62]. Consequently, our observations in the present study, indicating that 1,25(OH) $_2$ D $_3$

enhances the M2 polarization and A β uptake of human microglial cells in a TREM2-dependent manner, suggest a potential therapeutic option for the treatment of AD. However, more elaborate studies will be necessary to elucidate the precise mechanisms whereby vitamin D (1,25(OH) $_2$ D $_3$)-enhanced TREM2 expression is involved in facilitating the M2 polarization and A β uptake of HMO6 cells. In addition, in vitro studies using primary microglial cells and animal AD model studies are needed to evaluate the potential clinical applications of 1,25(OH) $_2$ D $_3$.

In conclusion, the findings of this study provide convincing evidence to indicate the potential protective effect of 1,25(OH) $_2$ D $_3$ on A β 42-treated human microglial cells by suppressing the expression levels of proinflammatory mediators, whilst enhancing the expression of anti-inflammatory mediators, which we suspect is associated with M2 polarization and A β uptake, plausibly mediated via an increase in cell surface TREM2 expression.

Abbreviations

1,25(OH) $_2$ D $_3$:	1,25-Dihydroxy vitamin D $_3$
AD:	Alzheimer's disease
TREM2:	Triggering receptor expressed on myeloid cells 2
TLR:	Toll-like receptor
VDR:	Vitamin D receptor.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

This study was partly based on the first author's master thesis submitted in 2021 to the Gachon University in Korea.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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