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Vitamin D Improves Cognitive Function and Modulates T_h17/T_{reg} Cell Balance After Hepatectomy in Mice

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Abstract—It is known that surgery-induced tissue damage activates the peripheral immune system resulting in the release of inflammatory mediators and cognitive impairment in aged mice. Vitamin D has been shown to have immunomodulatory function, but the molecular basis for it has not been well understood. In this study, we mainly investigated the efficacy and mechanism of vitamin D against postoperative cognitive dysfunction (POCD). The treatment of C57BL mice with vitamin D significantly preserves postoperative cognitive function, markedly inhibits surgery-induced interleukin (IL)-17, IL-6, transforming growth factor beta (TGF-β), and retinoic acid-related orphan receptor (RORγt) production, and obviously induces IL-10 and forkhead box p3 (Foxp3) expression. These findings indicate that vitamin D amelioration of POCD is, to a large extent, due to inhibit inflammatory CD4_T cell lineage, T helper 17 (T_h17) cells, accompanied with expansion in regulatory T cells (T_{reg} cells), a subset of CD4_T cells that are important in inhibiting inflammation. Our results suggest that T_h17 and T_{reg} cell imbalance may play a role in the development of POCD. Vitamin D is useful in the control of inflammatory diseases.

KEY WORDS: vitamin D; T_h17; T_{reg}; cognition.

INTRODUCTION

Postoperative cognitive dysfunction (POCD) is recognized as a complication associated with surgery [1–3]. Although a number of perioperative factors have been implicated, the pathogenic mechanisms of POCD remain largely unknown [4–6]. Our previous study suggests that cognitive impairment may be induced by surgically induced inflammation, particularly in the hippocampus, which plays a crucial role in processing memory [7, 8]. According to this hypothesis, surgery activates the host innate immune system resulting in a peripheral inflammation with elevated levels of proinflammatory cytokines such as interleukin (IL)-1β, tumor necrosis factor alpha (TNF-α), and IL-6. Using multiple signaling pathways, these peripheral cytokines [9, 10] are thought to affect the inflammatory state of the brain contributing to cognitive dysfunction. To date, adaptive immune responses and CD4_T cell subsets function have not been addressed in the pathogenesis of POCD, although CD4_T cell lineage, inflammatory T helper 17 (T_h17) cells, and regulatory T cells (T_{reg} cells), a subset of CD4_T cells that are important in inhibiting inflammation, have been reported in numerous inflammatory diseases, including systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis (MS) [11–14].

As a new subset of CD4_T cells, T_h17 cells were reported to have an important role in inducing local inflammation [15–17]. T_h17 cells produce proinflammatory molecules, IL-17A, IL-17 F, and IL-22, which act on tissue resident cells to promote inflammation. Transforming growth factor beta (TGF-β) and IL-6 are critical in the differentiation of T_h17 cells by inducing the transcription factors, retinoic acid-related orphan receptor (RORγt) [18], and IL-23 is also important to stabilize and maintain T_h17 cells [19, 20]. Of interest, a previous report indicated that vitamin D is able to prevent experimental autoimmune uveitis, partially because of its suppressive effect directly

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on T_h17 cells [21]. However, it is not known whether the effect can be observed in POCD.

Vitamin D is an essential nutrient that regulates calcium and phosphate transport and bone mineralization [22]. In recent years, however, vitamin D has been found to have a much broader range of actions, including regulation of cell differentiation, proliferation, and apoptosis [23]. The role of vitamin D in immune system is complex and diverse. The systemic or locally produced active form of vitamin D, 1,25-dihydroxyvitamin D3 ($1,25(OH)_2D_3$), can act directly on T cells or indirectly on dendritic cells to modulate T cell function [24–27]. Modulation of CD4_T cells by $1,25(OH)_2D_3$ is intriguing because several studies have demonstrated that $1,25(OH)_2D_3$ is capable of suppressing inflammation *in vivo* [21, 28–30].

Based on these properties, we are interested in that whether vitamin D can be developed as a potential treatment for POCD. The present study was conducted to test the hypothesis that vitamin D improves POCD via regulation of T_h17/T_{reg} cell balance.

MATERIALS AND METHODS

Animals

C57BL/6 (14 months old) female mice were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. and were housed under a 12-h light/dark cycle in microisolator cages contained within a laminar flow system to maintain a pathogen-free environment. The studies were conducted in accordance with the Animal Component of Research Protocol guidelines at the China Medical University.

Surgical Procedures

C57BL mice undergoing the water maze cognitive test were trained for five consecutive days and then randomly assigned to three groups: (1) control group (group C; $n=32$), mice received no intervention; (2) surgery group (group S; $n=32$), mice underwent partial hepatectomy under general anesthesia. For hepatectomy, the liver was exposed through a 1–2-cm midline abdominal incision. The left lateral lobes of the liver (approximately corresponding to 30 % of the organ) were excised. The wound was then infiltrated with 0.25 % bupivacaine and closed by sterile suture. (3) In surgery plus vitamin D group (group SD; $n=32$), mice were treated as in the S group, and they underwent surgery with administration of $1,25(OH)_2D_3$.

Treatment Protocol

$1,25(OH)_2D_3$ was stored at 4 °C. $1,25(OH)_2D_3$ treatment (50 ng) by intraperitoneal (i.p.) injection started at day 3 before operation and injection of $1,25(OH)_2D_3$ q.d. continued until the end of the experiment (days 3 and 7 after surgery). Surgery group was treated with an equal volume of saline.

Cognitive Test: Morris Water Maze

The Morris water maze (MWM) is a hippocampal-dependent test of spatial learning for rodents [31]. Mice underwent testing daily with three trials per day for five consecutive days before surgery and 7 days after surgery. Swimming distance, speed, and escape latency to the platform were recorded by video camera mounted to the ceiling, and digital images were analyzed by water maze software (HVS Image, UK). Additionally, on postoperative days 3 and 7, mice were subjected to a probe test in which the platform was removed and the mouse allowed swimming for 90 s. The time spent in the quadrant previously containing the submerged platform was recorded and represented an index of memory. Mice were sacrificed on postoperative days 3 and 7 after cognitive tests. Hippocampal tissues and spleens of mice in each group were quickly dissected and stored at −70 °C until they were used for messenger RNA (mRNA) and Western blot studies.

Pathological Examination

For histopathological studies, hippocampal tissues were dissected from female mice on day 3 after surgery, fixed in 10 % formalin in PBS, and embedded in a single paraffin block. The 6- to 10-μm-thick sections were stained with hematoxylin and eosin (H&E), and stained sections were evaluated for immune cell infiltration. For electron microscope (EM) studies, samples were fixed in 2.5 % glutaraldehyde in phosphate buffer pH 7.4 and postosmicated and processed routinely for EM. Sections of hippocampal tissues were examined using an H-600 transmission electron microscopy (TEM).

RNA Isolation and Quantitative Real-Time PCR

Total RNA was extracted from the hippocampus and spleens using an RNA isolation kit (Takara, Japan). Complementary DNA was prepared as recommended and used as the template for quantitative PCR. Levels of mRNA for IL-17, IL-6, TGF-β, IL-10, RORγt, and forkhead box p3 (Foxp3) from all groups were analyzed by real-time PCR. Real-time PCR was performed according to the

manufacturer's instructions. Specific primers are shown in Table 1. The amplification conditions were 8 min at 95 °C, followed by 45 cycles of 95 °C for 5 s, 60 °C for 34 s, and 72 °C for 15 s. The data were analyzed using the standard curve method, and the mRNA level of target gene for each sample was normalized against reference gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA. All values were expressed relative to the expression of GAPDH ($2^{-\Delta\Delta ct}$).

Western Blot Analysis

Hippocampal samples were homogenized in cold lysis buffer containing protease inhibitors and then centrifuged (12,000*g*, 10 min, 4 °C). Protein concentration of tissue samples was measured using the bicinchoninic acid (BCA) protein assay. Normalized protein samples were resolved by 10 % SDS-PAGE and then transferred onto polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). Membranes were blocked in 5 % skim milk, incubated with antibodies against IL-17 (1:100), IL-6 (1:100), TGF-β (1:100), and IL-10 (1:100) followed by HRP-labeled secondary antibodies (1:5000). Signals were detected by enhanced chemiluminescence (ECL; Amershan Biosciences, Chalfont St. Giles, UK) according to the manufacturer's instructions. β-Actin was used as a protein-loading control.

ELISA

The cytokine production from spleens was assessed with IL-17, IL-6, TGF-β, and IL-10 ELISA kits (R&D Systems) according to the manufacturer's instructions. A standard curve was generated using known amounts of the respective purified recombinant cytokines.

Statistics

The data are expressed as the mean±SEM. SPSS version 16 (SPSS, Chicago, IL, USA) was used for analysis. Statistical significance was determined by one-way analysis of variance (ANOVA). The Student-Newman-Keuls method was used for comparison between groups. A p value <0.05 was considered significant.

RESULTS

Vitamin D Ameliorates the Cognition of Hepatectomized Mice

To elucidate the effect of partial hepatectomy on learning and memory, we conducted the MWM test. It is particularly sensitive to hippocampus-dependent learning and memory formation. In our tests, mice in all groups were able to rescue themselves, and the escape latency gradually decreased over the five consecutive training days. On the fifth training day, the latency was reduced by 49, 44, and 50 % compared to the first day of training in the control group, surgery group, and surgery plus vitamin D group, respectively (Fig. 1a, $p>0.05$). On the first postoperative day, all three groups were able to locate the platform within a short time (24–31 s). However, the hepatectomized mice showed a significant increase in the escape latency time from 42 s on the second postsurgical day to 52 s on three postoperative days with a peak latency when compared with that of the control group at the corresponding time points (Fig. 1b, $p<0.05$). However, after $1,25(OH)_2D_3$ treatment, the escape latencies of the hepatectomized mice decreased obviously (Fig. 1b, $p<0.05$). As expected, the swimming speed was not significantly

Table 1. Specific Primer Sequence for Real-Time PCR

Gene	Forward (5′-3′)	Reverse (5′-3′)
IL-17	CTGTGTCTCTGATGCTGTTGC	GTGGAACGGTTGAGGTAGTCT
IL-6	ACTTCCATCCAGTTGCCTTCTT	TCATTTCCACGATTTCCCAGA
TGF-β	GCAACAATTCCTGGCGTTACCT	GAAAGCCCTGTATTCCGTCTCC
IL-10	CCAGTTTTACCTGGTAGAAGTGATG	TGTCTAGGTCCTGGAGTCCAGCAGACTCAA
RORγt	CAGTATGTGGTGGAGTTTGCCAA	TGTAGGCCCTGCACATTCTGAC
Foxp3	CAGCTCTGCTGGCGAAAGTG	TCGTCTGAAGGCAGAGTCAGGA
GAPDH	TGTGTCCGTCGTGGATCTGA	CCTGCTTCACCACCTTCTTGA

IL interleukin, *TGF*-β transforming growth factor beta, *RORγt* retinoic acid-related orphan receptor, *Foxp3* forkhead box p3, *GAPDH* glyceraldehyde 3-phosphate dehydrogenase

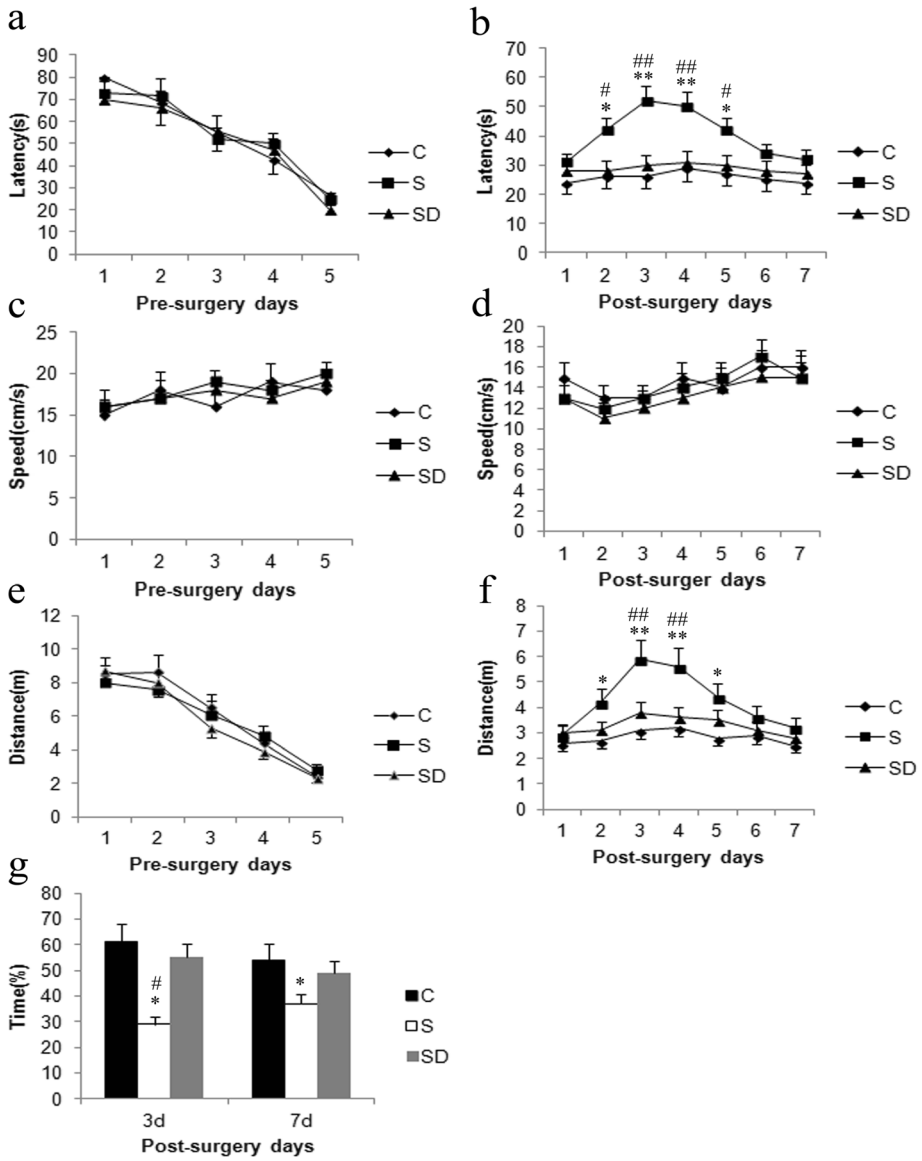

Fig. 1. Cognitive impairment induced by hepatectomy surgery in old mice. The Morris water maze was used to train mice for their memory formation for 5 days before experiments and to assess their memory formation changes after treatments for 7 days. Escape latency obtained from the Morris water maze test throughout the experiments (**a**, **b**). Swimming speed obtained from the Morris water maze test throughout the experiments (**c**, **d**). Swimming distance obtained from the Morris water maze test throughout the experiments (**e**, **f**). The probe trial test (**g**). *C* control group, *S* surgery group, *SD* surgery plus vitamin D group. Data are represented as means±SEM; $n=12$. Means with *$p<0.05$; **$p<0.01$ vs. control group at the corresponding time point. #$p<0.05$; ##$p<0.01$ vs. surgery plus vitamin D (50 ng) group at the corresponding time point.

different among groups at any corresponding time points (Fig. 1c, d, $p>0.05$), and the pattern of change in distance swam was the same as that of the latency (Fig. 1e, f, $p<0.05$). Our probe tests revealed that the time spent in the quadrant with the previously located hidden platform was significantly less in the hepatectomized mice (29 %) when compared with the control mice (61 %) on day 3 after surgery (Fig. 1g, $p<0.05$). $1,25(OH)_2D_3$-treated mice undergoing probe trials showed significant difference to those in the surgery group (Fig. 1g, $p>0.05$). The above results indicate that the hepatectomy induced a significant cognitive impairment and that vitamin D treatment before and after surgery attenuated the surgically induced cognitive impairment.

Partial Hepatectomy-Induced Hippocampus Pathological Injury Could Be Rescued by Vitamin D

To further study the effect of vitamin D on the pathogenesis of hippocampus inflammation, we examined the effect of vitamin D on POCD *in vivo*. Mice from surgery group and $1,25(OH)_2D_3$ group were sacrificed on day 3 after surgery. Hippocampus sections from mice were stained with H&E to assess tissue inflammation. As shown in Fig. 2, the surgery mice showed prominent inflammatory cell infiltration in the hippocampus (Fig. 2a). Conversely $1,25(OH)_2D_3$-treated mice developed mild inflammatory infiltration (Fig. 2b). To evaluate the ultrastructure of the hippocampus with the treatment of $1,25(OH)_2D_3$, hippocampal tissues were examined by TEM at day 3 after surgery. Compared with $1,25(OH)_2D_3$-treated mice, severe chromosome aberrations, dilatate, and widened synaptic clefts as well as disappeared mitochondriales crista were observed in the hippocampus of surgery mice. Representative slides exemplifying hippocampus lesions are presented in Fig. 2c, d.

Vitamin D Inhibits IL-17 mRNA and Protein Expressions in Hepatectomized Mice

IL-17 is associated with the development of inflammatory diseases. To investigate the effect of hepatectomy and/or vitamin D administration on the production of IL-17, we measured hippocampal and splenic levels of IL-17. Injury caused by hepatectomy increased the production of IL-17 mRNA and protein both in the hippocampus and spleen at 3 days after surgery (Fig. 3c, $p<0.05$). In $1,25(OH)_2D_3$-treated animals, the expressions of IL-17 mRNA and protein were significantly decreased in the hippocampus and spleen at 3 days after surgery in the hepatectomized mice compared to hepatectomized animals without the treatment of $1,25(OH)_2D_3$ (Fig. 3a–c, $p<0.05$).

Vitamin D Suppresses the Cytokines Associated with T_h17 Differentiation in Hepatectomized Mice

T_h17 cells produce proinflammatory molecule IL-17. The differentiation of T_h17 cell has been associated with various inflammatory cytokines and transcription factors, including IL-6, TGF-β, and RORγt. To determine the mechanisms involved in vitamin D suppression of T_h17 responses in POCD, the mRNA and protein levels of IL-6, TGF-β, and RORγt have been identified and quantified in the hippocampus and spleen. As data have shown, expressions of IL-6 (Fig. 4a–c), TGF-β (Fig. 4d–f), and RORγt (Fig. 4g) were

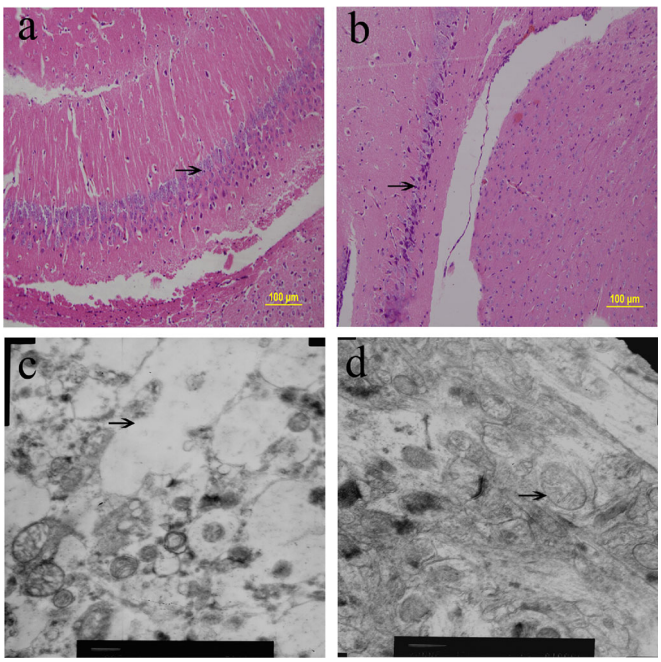

Fig. 2. Vitamin D inhibited the inflammatory cell infiltration and ultrastructure damage in the hippocampus of the hepatectomized mice. Mice from surgery group and surgery plus vitamin D-treated (50 ng) group were sacrificed on day 3 after hepatectomy surgery. H&E staining demonstrated areas of inflammation, and hippocampal ultrastructure was examined by TEM in surgery (**a**, **c**) and surgery plus vitamin D (50 ng) group (**b**, **d**). Original magnification H&E×200 and TEM×8,000; $n=4$.

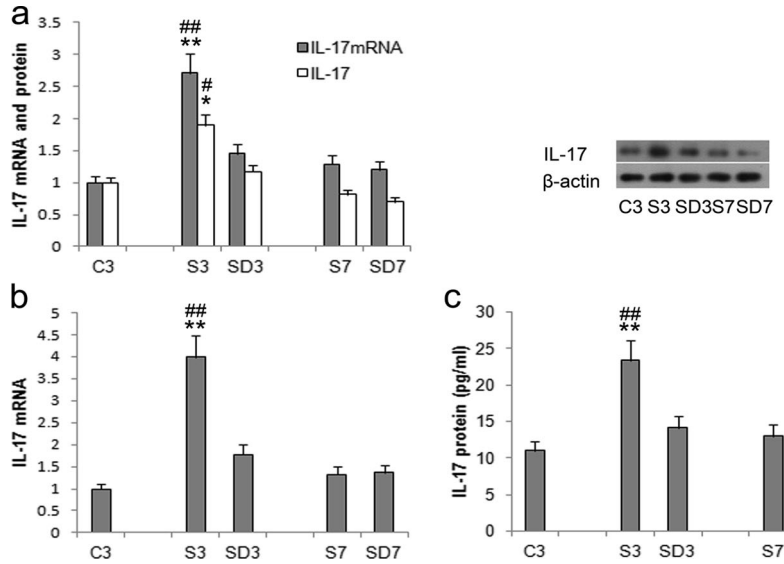

Fig. 3. Effect of vitamin D on expressions of IL-17 mRNA and protein in hepatectomized mice. Expression of IL-17 mRNA was detected by real-time PCR; its protein from the hippocampus was measured by Western blotting, and its protein from splenocyte was assayed by ELISA. The measurements were made at 3 days in control mice (*C3*), at 3 and 7 days after hepatectomy surgery (*S3*, *S7*), or at 3 and 7 days after hepatectomy surgery plus vitamin D (50 ng) i.p. (*SD3*, *SD7*) both in the hippocampus (**a**) and spleen (**b**, **c**). Data are represented as means±SEM; $n=12$. Means with $^{*}p<0.05$; $^{**}p<0.01$ vs. control group at the corresponding time point. $\#p<0.05$; $\#\#p<0.01$ vs. surgery plus vitamin D group at the corresponding time point.

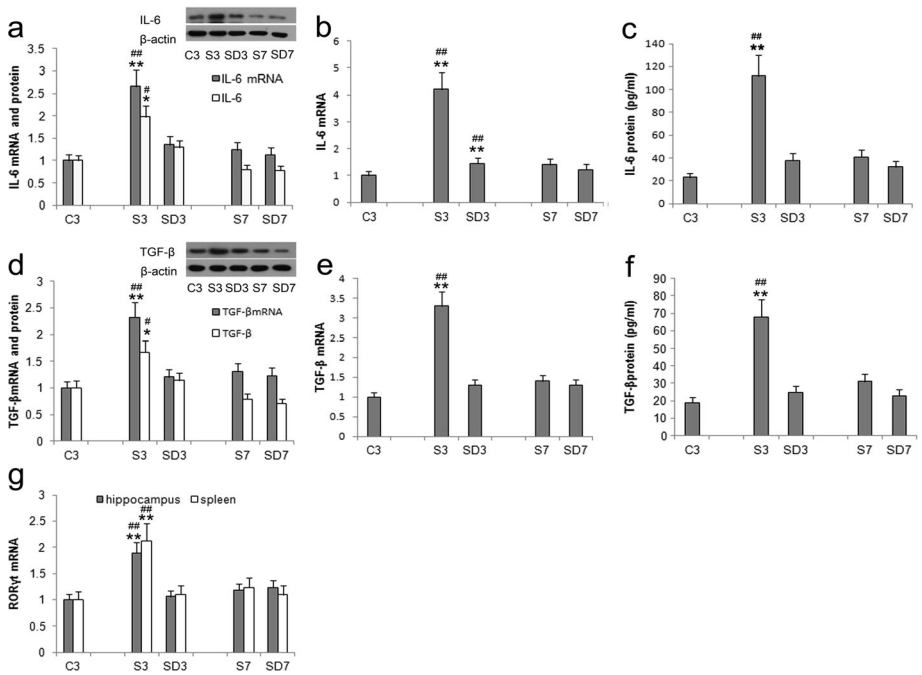

Fig. 4. Vitamin D suppressed IL-17 differentiation-related cytokines and transcription factor expressions in hepatectomized mice. Mice from control group, surgery group, and vitamin D-treated (50 ng) group were sacrificed on days 3 and 7 after hepatectomy surgery. The mRNA levels of cytokines in the hippocampus and spleen were quantified by real-time PCR; their protein levels from the hippocampus were measured by Western blotting, and their protein levels from splenocyte were assayed by ELISA. The expression levels of IL-6 (**a**–**c**), TGF-β (**d**–**f**), and RORγt (**g**) were significantly reduced in vitamin D-treated mice compared with the surgery mice on day 3 both in the hippocampus and spleen. Data are represented as means±SEM; $n=12$. Means with $^{*}p<0.05$; $^{**}p<0.01$ vs. control group at the corresponding time point. $\#p<0.05$; $\#\#p<0.01$ vs. surgery plus vitamin D group at the corresponding time point.

significantly reduced in the hippocampus and spleen in $1,25(OH)_2D_3$-treated mice compared with the surgery mice at 3 days after surgery ($p<0.05$). These results suggested that vitamin D can suppress the expressions of inflammatory cytokines and signal transducers associated with differentiation and production of T_h17 cells.

Vitamin D Induces T_{reg} Cell Expansion in Hepatectomized Mice

Foxp3 is a transcription factor involved in the development and function of T_{reg} cells [32]. The effect of vitamin D on the expression of Foxp3 and IL-10 in both the periphery and the inflamed hippocampus from hepatectomized mice was examined. IL-10 is an anti-inflammatory cytokine and mainly secreted from T_{reg} cells, and the increased IL-10 mRNA and protein expression were observed in both the hippocampus and spleen in $1,25(OH)_2D_3$-treated mice compared with the surgery mice at 3 and 7 days after surgery (Fig. 5a–c, $p<0.05$). We also observed a significant reduction of Foxp3 mRNA expression in the hippocampus and spleen in the surgery mice at 3 days after surgery and up to postoperative day 7 (Fig. 5d, $p<0.05$). $1,25(OH)_2D_3$ treatment induced a significant expansion of T_{reg} cell within the hippocampus and spleen compared with the surgery mice (Fig. 5d, $p<0.05$).

DISCUSSION

Several recent rodent models have demonstrated surgery-induced neuroinflammation and POCD. Mice undergoing fear-conditioning tests in a model of tibial surgery had hippocampal memory impairment with central IL-1β and TNF-α [33, 34]. A rat model of splenectomy using the Y maze memory test showed brain IL-1β mRNA levels associated with temporary memory impairment [8]. Following minor abdominal surgery, aged mice showed an exaggerated neuroinflammatory response associated with brain IL-1β mRNA level and had reduced cognitive flexibility in the MWM [2]. After partial hepatectomy, aged rats showed memory impairment in the MWM, and brain cytokine increases [7]. Although many cytokines increased in the brain of POCD mice, little is known about CD4_T subset-related cytokines, especially inflammatory IL-17 involvement in POCD.

This study provides evidence for the first time that short-term cognitive impairment is associated with enhanced production of T_h17 cells and inhibition of T_{reg} cells in the hippocampus after liver surgery in old mice. Our data also indicate that IL-17 expression in the hippocampus could be due to the increased levels of inflammatory cytokines and transcription factors, including IL-6, TGF-β, and RORγt induced by surgery. The surgery-induced cognitive dysfunction, where T_h17 cells increase and T_{reg} cells decrease, was abolished by the administration of vitamin D.

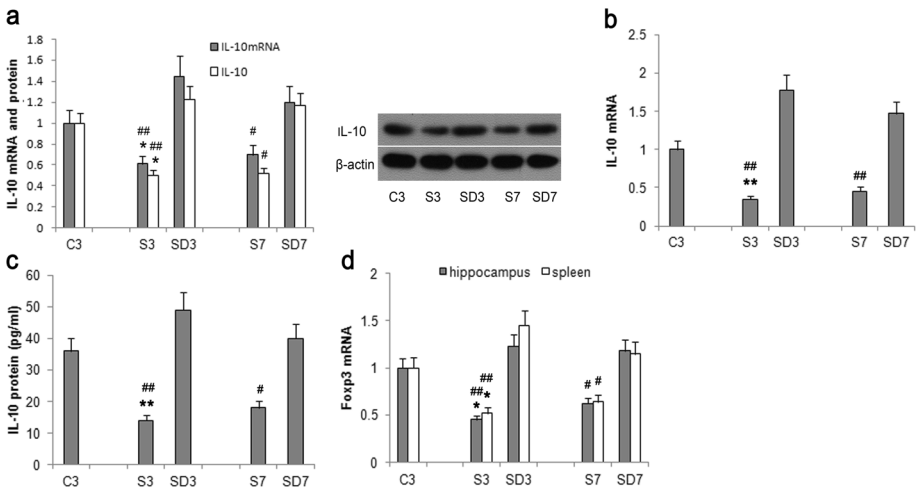

Fig. 5. Vitamin D treatment induced T_{reg} cell expansion in hepatectomized mice. We detected the T_{reg} cell marker Foxp3 mRNA expression from the hippocampus and spleen on days 3 and 7 after hepatectomy surgery. We also examined the T_{reg} cell-related cytokine IL-10 mRNA by real-time PCR, its protein level from the hippocampus by Western blot, and its protein level from splenocyte by ELISA assay on days 3 and 7 after hepatectomy surgery. Vitamin D therapy (50 ng) induced a substantial increase in IL-10 (**a** hippocampal IL-10; **b**, **c** splenic IL-10) and Foxp3 (**d**) expression on days 3 or 7 in the hippocampus and spleen after surgery (**a**–**c**). Data are represented as means±SEM; $n=12$. Means with $^*p<0.05$; $^{**}p<0.01$ vs. control group at the corresponding time point. $\#p<0.05$; $\#\#p<0.01$ vs. surgery plus vitamin D group at the corresponding time point.

Previous studies have suggested a supporting role for vitamin D in reducing the risk of MS [35–37]. In addition, $1,25(OH)_2D_3$ is known to inhibit the development of experimental autoimmune encephalomyelitis (EAE) [38, 39], and recent studies have correlated a suppressive effect of $1,25(OH)_2D_3$ on T_h17 with the prevention of EAE [40]. $1,25(OH)_2D_3$ has also been shown to inhibit IL-17A production in T cells from MS patients [41] and from patients with early rheumatoid arthritis [42]. The ability of $1,25(OH)_2D_3$ to prevent experimental autoimmune uveitis and to reduce colitis in a mouse model has also been correlated to a suppression of IL-17A induction [30, 43]. These results reveal that $1,25(OH)_2D_3$ modulates T_h17-mediated inflammation. Our findings are the first to correlate the reversal of cognition in surgery mice by $1,25(OH)_2D_3$ with reduced IL-17. Vitamin D treatment not only abrogates the expression of peripheral IL-17 but also inhibits IL-17 production within the hippocampus in surgery mice. Our data indicate that vitamin D ameliorates POCD by inhibiting the IL-17 expression or the T_h17 cell production. However, it is still unknown by what mechanism vitamin D suppresses expansion of the T_h17 cell subset in surgery animals. Since proinflammatory cytokines determine CD4_T cell differentiation and to further address the mechanisms involved in the inhibition of T_h17 cell by vitamin D, we examined the crucial cytokines involved in the differentiation and production of T_h17 cells. Studies have shown that T_h17 cell differentiation is independent of IL-23 and is induced by TGF-β plus IL-6 [44, 45], but maintenance of proinflammatory T_h17 cells requires the presence of IL-23 [20, 46, 47]. Both *in vitro and in vivo* differentiation of T_h17 cell lineage is required in the activation of STAT3, which is activated by IL-6, and then upregulation of RORγt [44, 48] that is served as the master switch of the differentiation of T_h17 cell [49–52]. Our data discover that vitamin D obviously inhibits IL-6, TGF-β, and RORγt production both in the peripheral system and in the central nervous system (CNS), suggesting that the inhibition of IL-6, TGF-β, and RORγt is a mechanism of the regulation of cognition in surgery mice by vitamin D. Another mechanism may be related to a direct modulation of inflammatory environment; a less severe inflammatory environment in peripheral lymphoid tissue as well as in the CNS after vitamin D therapy may prevent significant T_h17 migration into the CNS, thereby inducing less severe CNS pathology.

In this study, we also show that vitamin D upregulates Foxp3 at the transcriptional level. Foxp3, a transcription factor involved in the development and function of T_{reg} cells, a subset of CD4_T cells that are important in inhibiting inflammation and in suppressing autoimmune processes [32], has recently been reported to negatively regulate both IL-17 and IL-2 transcription. $1,25(OH)_2D_3$ has been reported to result in an enhancement of T_{reg} cells and an inhibition of T_h17 cells [53]. To examine whether induction of Foxp3 may be an additional mechanism involved in the improvement of POCD by $1,25(OH)_2D_3$, we detected Foxp3 at the transcriptional level by real-time PCR. Our results indicate that $1,25(OH)_2D_3$ obviously induces Foxp3 transcription not only in the peripheral system but also in the CNS, suggesting that the induction of T_{reg} is a mechanism of the regulation of cognition in surgery mice by $1,25(OH)_2D_3$. Additionally, previous studies have indicated a role for IL-10 in the effect of $1,25(OH)_2D_3$ on EAE [54], and our *in vivo* data also demonstrated that $1,25(OH)_2D_3$ represses the expression of IL-17 concomitant with the upregulation of IL-10, a T_{reg} cell-associated and inhibiting inflammation cytokine, keeping the balance of T_h17/T_{reg} cells, or their specific cytokines IL-17/IL-10 balance.

Thus, multiple mechanisms are involved in the amelioration of POCD by $1,25(OH)_2D_3$. The regulation of POCD along the T_h17 and T_{reg} axes and the key role of vitamin D deficiency as a susceptibility factor for this disease add to the relevance of this study. These studies result in new concepts with regard to the mechanisms involved in the interaction of the vitamin D endocrine system and the immune system and provide a foundation for trials involving $1,25(OH)_2D_3$ or $1,25(OH)_2D_3$ analogs for targeting T_h17 immunity.

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