Effect of BDNF on Differentiation of Circulating Th17 and Treg Cells in SLE Patients and Exploration of Signal Transduction Pathways

Bailing Tian
The First Hospital of China Medical University: The First Affiliated Hospital of China Medical University

Xiaoyu Hou
The First Affiliated Hospital of China Medical University

Mengmeng Zhao ( cmu2005meng@126.com)
The First Affiliated Hospital of China Medical University

Research Article

Keywords: Brain-derived neurotrophic factor, systemic lupus erythematosus, Treg cells, Th17 cells, signal transduction pathways

DOI: https://doi.org/10.21203/rs.3.rs-175686/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Circulating brain-derived neurotrophic factor (BDNF) is mainly derived from lymphocytes. The serum BDNF level in SLE is decreased, and BDNF may be involved in the pathogenesis of systemic lupus erythematosus (SLE). Our aim is to determine whether BDNF affects the differentiation of CD4+T cells into regulatory T (Treg) or T helper 17 (Th17). 30 patients were selected. TGF-β and IL-6 were added to induce differentiation of Treg and Th17. After co-cultured with BDNF, the percentages of CD4+CD25+CD127low and CD4+IL-17A+ were detected by Flow cytometry, and the expression of Foxp3mRNA and RORγtmRNA were detected by Rt-PCR. Under the condition of Th17 and Treg polarization, after co-cultured with BDNF and TrkB IgG, the phosphorylation of Akt, mTORC1 and ERK1/2 were detected by western-blot, the percentages of CD4+CD25+CD127low and CD4+IL-17A+ were detected by Flow cytometry. Under the condition of Th17 polarization, with the increase of BDNF concentration (60ng/ml, 120ng/ml, 350ng/ml), the percentages of CD4+IL-17A+ and the expression of RORγtmRNA were decreased (p<0.01; p<0.001). Under the condition of Treg polarization, the percentages of CD4+CD25+CD127low and the expression of Foxp3mRNA were increased (p<0.001). Regardless of Th17 or Treg polarization, the phosphorylation of Akt, mTORC1 and ERK1/2 in the BDNF group were reduced (p<0.001), and the phosphorylation of Akt, mTORC1 in the TrkB IgG group were enhanced (p<0.001). BDNF down-regulates the differentiation of Th17 and promotes the differentiation of Treg in SLE through inhibiting the activation of PI3K-Akt-mTORC1 axis and ERK1/2 pathway. BDNF could play a certain role in maintaining the balance of Treg/Th17 ratio in SLE.

1. Introduction

Initial CD4+T cells differentiate into regulatory T (Treg) and T helper (Th). Treg are characterized by sustained expression of CD25+, transcription factor Foxp3, and low expression of CD127, which secrete cytokine transforming growth factor-β (TGF-β) and play an essential role in maintaining immunologic homeostasis and preventing the occurrence of autoimmune diseases[1]. Th17, a distinct subset of Th cells characterized by expression of the transcription factor RORγt. Particularly, Th17 plays a pivotal role in the initiation and development of autoimmunity, which secretes a profile of potent pro-inflammatory cytokines upon certain stimulation, including interleukin 17 (IL-17), IL-2[2] etc. Th17 are involved in the pathogenesis of many autoimmune diseases[3]. TGF-β is a cytokine shared during the differentiation of Th17 and Treg. However, the presence of other pro-inflammatory cytokines during the activation of lymphocytes further regulates the differentiation of these cells. Under the coexistence of IL-6 and TGF-β, CD4+T cells differentiate into Th17, while in the absence of IL-6, TGF-β promotes the differentiation of Treg[4,5]. Therefore, the balance between pro-inflammatory cytokines and anti-inflammatory cytokines is a key factor in the differentiation of CD4+T cells[6]. It was reported that under the co-stimulation of TCR and CD28, the activation, survival and proliferation of Th17 is up-regulated through the activation of PI3K-Akt-mTORC1 axis[7-9]. Activation of the MAPK/ERK pathway also promotes the differentiation of Th17(Sup.1)[10].
Systemic lupus erythematosus (SLE) is a heterogeneous chronic inflammatory autoimmune disorder characterized by the loss of immune tolerance to autoantigen, continuous production of pathogenic autoantibodies and deposition of immune complexes in different organs. Increasing evidences suggested that defects in the number or function of Treg in SLE can lead to an increasing activity in Th17[3,11-13]. Th17 participate in the attack on target cells or tissues through the production of excessive pro-inflammatory cytokines, which ultimately leads to the damage of target tissues[14]. Pro-inflammatory cytokines such as IL-6, IL-8, IL-17, and IFN-γ were significantly elevated in the serum and in cerebrospinal fluid of neuropsychiatric lupus erythematosus (NPSLE) patients[15].

Brain-derived neurotrophic factor (BDNF) plays an essential role in promoting the growth, differentiation and survival of neurons[16]. In addition, BDNF has been found in peripheral blood. Lymphocytes and vascular endothelial cells are the main sources of BDNF[17,18]. BDNF can promote the proliferation of T lymphocytes and has an anti-apoptosis effect on T cells[19,20]. However, the relationship between BDNF and T lymphocytes is unclear. We have reported that serum BDNF in SLE patients is significantly decreased. The serum BDNF level is positively related with SLEDAI scores[21,22]. We speculated that BDNF may involve in the pathogenesis of SLE. Our aim is to future determine whether BDNF can affect the differentiation of peripheral Treg and Th17 in SLE, and to explore the signal transduction pathway.

2. Methods

2.1 Subjects

We recruited thirty naïve SLE patients from March to September 2019 who were definitely diagnosed after hospitalization in the Rheumatology Department of the First Affiliated Hospital of China Medical University. 15ml EDTA anticoagulant whole blood was collected from the patients after overnight of fasting. Diagnosis of SLE based on the Revised criteria from Systemic Lupus International Collaborating Clinics (SLICC) and American College of Rheumatology[23]. SLE disease activity index (SLEDAI) scoring system was used to evaluate disease activity, and the patients with ≥5 scores were selected[24]. The study was approved by the Ethics Committee of the First Affiliated Hospital of China Medical University (No. 2018-214-3) and conducted in accordance with the declaration of Helsinki. Informed consent and signature will be obtained after detailed description and explanation of the study to all subjects.

Exclusion criteria: (1) combination of other autoimmune diseases; (2) acute or chronic infection, including hepatitis virus or human immunodeficiency virus infection; (3) tumors; (4) endocrine and metabolic diseases; (5) end-stage hepatic and renal insufficiency; (6) drug abuse; (7) have taken anti-anxiety and depression drugs.

2.2 Preparation of peripheral blood mononuclear cells
EDTA anticoagulant venous whole blood was layered on a Ficoll-Paque density gradient (GE, USA) and centrifuged at room temperature according to the manufacturer’s recommended protocol. The peripheral blood mononuclear cell (PBMC) layer was collected, washed with 1×PBS twice and then resuspended for 107/ml density cells. Cell viability was greater than 95%, as determined by trypan blue exclusion assay under the optical microscope.

### 2.3 Isolation of CD4+T lymphocytes

PBMCs were resuspended in RPMI-1640 medium (HyClone, Logan, UT, USA) containing 10% heat-inactivated fetal calf serum, 40 umol/l L-glutamine, 100 U/ml penicillin, 100 U/ml streptomycin at room temperature. CD4+T cells were purified by positive selection with immunomagnetic beads using human CD4+T-cell isolation kit according to the manufacturer's instructions (130-045-101, Miltenyi Biotec, Germany). Starting with 1-2×10^7 PBMCs, 50-100×10^6 CD4+T cells were typically isolated. The purity of CD4+T cells was more than 90% (Sup.2).

### 2.4 Cell culture

24-well plate was coated with 1×PBS and anti-CD3 (2ug/ml) for 4-6h. CD4+T lymphocytes were mixed with anti-CD28 (2ug/ml). TGF-β (2.5ng/ml) was added to induce differentiation of Treg cells, and TGF-β (2.5ng/ml) + IL-6 (25ng/ml) were added to induce differentiation of Th17 cells. Then these cells were treated with different concentrations of human recombinant BDNF (60ng/ml, 120ng/ml, 350ng/ml) respectively, and cultured at 37°C in a 5% CO2 incubator for 72 hours.

### 2.5 Cell surface/intracellular staining and flow cytometry analysis

PBMCs were resuspended in PBS containing 1% bovine serum albumin. For the staining of surface antigens, cells were incubated with monoclonal antibodies or their isotype control in the dark for 30 min on ice. Intracellular staining of Foxp3 and IL-17A was performed for fixation and permeabilization according to the manufacturer's instructions. The antibodies used for the surface or intracellular marker analysis include FITC-conjugated CD4 antibody (11-0049-42, eBioscience, USA), PE-conjugated CD25 antibody (12-0259-42, eBioscience, USA), APC-conjugated CD127 (351315, Biolegend, USA) and isotype for CD127 (Mouse IgG1 kappa isotype control, 17-4714-82, eBioscience, USA), PE-conjugated IL-17A (12-7179-42, eBioscience, USA). FACSort flow cytometry (FACSCalibur, BD Biosciences) was used to evaluate the expression of cell surface and intracellular markers in all samples, and FlowJo v10 software (Tree Star, Ashland, USA) was used for data analysis.

### 2.6 Quantitative PCR
The cells were cultured and harvested at the indicated times. Trizol Reagent (RP1001, BioTeke, Beijing) was used to isolate total RNA from the cultured cells above according to the manufacturer's instructions. The RNA samples were reverse transcribed using a PrimeScript RT reagent Kit (PR6502, BioTeke, Beijing) to obtain the corresponding cDNA, and finally performed real-time fluorescence quantitative analysis. Quantitative detection of gene transcripts was performed using the following primers: Foxp3, Forward: 5'TGACCAAGGCTTCATCTGTG3', Reverse: 5'GAGGACTCTGGGAATGTGC3', 179bp; RORγt, Forward: 5'CTGTAACGCGGCTACTCCT3', Reverse: 5'GGCTGTCCCTCTGCTTCTTG3', 161bp; β-actin, Forward: 5'GGCACCCAGCACAATGAA3', Reverse: 5'TAGAAGCATTTGCGGTGG3', 168bp. Reaction conditions were as follows: 94℃ for 5 min, followed by 40 cycles of 94℃ for 10s, 60℃ for 20s and 72℃ for 30s, then 72℃ for 150s, 40℃ for 90s, 25℃ for 60s. TP800 real-time fluorescent quantitative PCR instrument (TaKaRa, Japan) was used for amplification, and the results were quantitatively analyzed by 2^-ΔΔCT.

2.7 Western blot

The cultured cells were rinsed with phosphate-buffered saline (PBS) and lysed in 1% Triton lysis buffer. Total proteins were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrophoretically transferred to nitrocellulose membranes. The membranes were blocked with 5% skimmed milk in TBST buffer at room temperature for 1h and incubated overnight at 4℃ with the primary antibodies. After the appropriate secondary antibodies were added for 45 min, the proteins were detected using an enhanced chemiluminescence reagent (SuperSignal Western Pico Chemiluminescent Substrate; Pierce, USA) and the optical density of the target strip was analyzed by Gel-Pro-Analyzer.

2.8 Statistical analysis

All the data were presented as a mean ± standard deviation. We used the t-test for statistical analysis for parametric data and the Mann-Whitney U test for non-parametric data. One-way analysis of variance (ANOVA) was used when there were more than two groups. We performed statistical analyses with SPSS 17.0 software and GraphPad Prism 5.0 (GraphPad Software, USA) and considered a P value less than 0.05 as significance.

3. Results

3.1 Clinical characteristics of the subjects

The mean age of individuals was 29.7 ± 11.6 years old (mean ± SD), age range (18 to 48 years old), and the average course of disease was 3.1 months. All patients were naïve SLE who without the treatment of hormones or immunosuppressive agents. The SLEDAI scores of all patients were ≥ 5 points. Lung involvement included interstitial pneumonia, pleural effusion and pleural disease. Among SLE patients
with neuropsychiatric symptoms, two was epilepsy, one was stroke and one was acute confusional state (Table 1).

### Table 1
Clinical features and manifestations of SLE patients

| characteristics                        | SLE (n = 30) |
|----------------------------------------|--------------|
| Age, mean (S.D.), years                | 29.7 (11.6)  |
| Male/Female, n                         | 3/27         |
| Naïve patients, n                      | 30           |
| Disease duration, median, months       | 3.1          |
| Clinical manifestations, n (%)         |              |
| Malar rash                             | 12 (40)      |
| Photosensitivity                       | 9 (30)       |
| Oral ulcer                             | 11 (36.7)    |
| Arthritis/Arthralgia                   | 8 (26.7)     |
| Autoimmune hemolytic anemia            | 12 (40)      |
| Leukopenia                             | 19 (63.3)    |
| Lymphopenia                            | 28 (93.3)    |
| Thrombocytopenia                       | 16 (53.3)    |
| LN                                     | 19 (63.3)    |
| NPSLE                                  | 4 (13.3)     |
| Lung involvement                       | 6 (20)       |
| SLEDAI                                 | All ≥ 5, 15 ± 6 |

LN: lupus nephritis; NPSLE: neuropsychiatric lupus erythematosus; SLEDAI: SLE disease activity index.

### 3.2 Effect of BDNF on proliferation and differentiation of Th17 in CD4 + T lineage

After co-cultured for 72 hours, the proportion of CD4 + IL-17A + and the expression of RORγtmRNA in the TGF-β + IL-6 group were increased significantly. Figure 1A enumerated the flow diagram that the proportion of CD4 + IL-17A+. The result showed that under the condition of Th17 polarization, the
proportion of CD4 + IL-17A + and the expression of RORγt mRNA gradually decreased after the addition of different concentration BDNF (60ng/ml, 120ng/ml, and 350ng/ml) (p < 0.01, p < 0.001 respectively. Figure 1B and Fig. 1C). Our result revealed that BDNF inhibited the proliferation and differentiation of Th17 by down-regulating the expression of RORγt mRNA in CD4 + T lineage, and the effect was augmented with the increase of BDNF concentration.

3.3 Effect of BDNF on proliferation and differentiation of Treg in CD4 + T lineage

The proportion of CD4 + CD25 + CD127\textsuperscript{low} in the TGF-\beta group was increased significantly. Figure 2A enumerated the flow diagram that the proportion of CD4 + CD25 + CD127\textsuperscript{low}. The result showed that under the condition of Treg polarization, the proportion of CD4 + CD25 + CD127\textsuperscript{low} was gradually increased after the addition of 120ng/ml and 350ng/ml BDNF (p < 0.001, Fig. 2B), but the difference was not significant (p = 0.181) in low-dose BDNF group (60ng/ml). The expression of Foxp3 mRNA was gradually increased after addition of BDNF (60ng/ml, 120ng/ml, and 350ng/ml) (p < 0.001, Fig. 2C). Our result revealed that BDNF promoted the proliferation and differentiation of Treg by up-regulating the expression of Foxp3 mRNA in CD4 + T lineage, and the effect was augmented with the increase of BDNF concentration.

3.4 Study of the signal transduction pathway mediated by BDNF during the development of Th17 and Treg

3.4.1 BDNF inhibits the proliferation and differentiation of Th17 through PI3K-Akt-mTORC1 axis and ERK1/2 pathway

Under the condition of Th17 polarization, the phosphorylation of Akt, mTORC1 and ERK1/2 was detected by western-blot. The results were showed in Fig. 3A and B. TGF-\beta + IL-6 group was the positive control, and the phosphorylation of Akt, mTORC1 and ERK1/2 was significantly enhanced. Compared with the positive control, the phosphorylation of Akt, mTORC1 and ERK1/2 in the TGF-\beta + BDNF group was reduced (p < 0.001), while there was no significant difference in the phosphorylation of Akt in the TrkB IgG pretreatment group. Although the phosphorylation of mTORC1 in the TrkB IgG group was decreased compared with the positive control, the phosphorylation was also significantly enhanced when compared with the TGF-\beta + IL-6 + BDNF group (p < 0.001). Our result demonstrated that BDNF down-regulated the development of Th17 by inhibiting the activation of PI3K-Akt-mTORC1 axis and ERK1/2 pathway. Meanwhile, the proportion of Th17 was consistent with the activation of PI3K-Akt-mTORC1 axis (Fig. 3C).

3.4.2 BDNF upregulates the proliferation and differentiation of Treg through PI3K-Akt-mTORC1 axis and ERK1/2 pathway
Under the condition of Treg polarization, the results were showed in Fig. 4A and B. The phosphorylation of Akt, mTORC1 and ERK1/2 was significantly reduced in the TGF-β group. Compared with the TGF-β group, the phosphorylation of Akt and mTORC1 in the TGF-β + BDNF group was reduced (p < 0.001, respectively), although the phosphorylation of ERK1/2 was also reduced in the TGF-β + BDNF group, but the difference was not significant (p = 0.086), while there was no significant difference in the phosphorylation of Akt and mTORC1 in the TrkB-IgG pretreated group. Our result demonstrated that BDNF up-regulated the proliferation and differentiation of Treg by inhibiting the activation of PI3K-Akt-mTORC1 axis, probably also including the inhibition of the ERK1/2 pathway. Figure 4C showed the proportion of Treg.

4. Discussion

For the first time we studied the effect of BDNF on the proliferation and differentiation of peripheral blood CD4+ T cells into Th17 and Treg in SLE patients. The results showed that BDNF inhibited the development of Th17 and up-regulated the development of Treg. In addition, we also explored the signal transduction pathway, which indicated that BDNF affect the development of Th17 and Treg through inhibiting the activation of PI3K-Akt-mTORC1 axis and ERK1/2 pathway.

Recently the theory of neural-immune network has been proposed[25,26]. There were several reports on serum BDNF level in NPSLE patients, suggesting that BDNF was associated with the disease activity and brain parenchymal injury of NPSLE[27]. We also reported BDNF level was significantly decreased in serum of SLE[21]. However, the relationship between BDNF and T cell subpopulation is ambiguous.

Aberrant immune response of T lymphocytes plays a crucial role in the pathogenesis of SLE(Sup.3)[28]. Increasing quantity of T helper cells attributes to excessive secretion of inflammatory cytokines. It was reported that there existed an imbalance of cytokines in the serum of SLE[14,29], with elevated cytokines such as IL-4, IL-6, IL-17A. Such imbalance is not limited to SLE flares, but is the hallmark of the disease, since also patients with quiescent disease display a TH17/Treg ratio favoring Th17. Amount of evidences demonstrated that Th17 cells and IL-17A play important roles in the pathogenesis of SLE[28,30]. Differentiation of Th17 and Treg is interrelated and restricted. RORγt and Foxp3 are the key transcription factors for the differentiation of Th17 and Treg[4-6,31]. TGF-β can induce the expression of RORγt and Foxp3 simultaneously, but the interaction between Foxp3 and RORγt inhibits the action of RORγt[5]. Only in the presence of IL-6 the inhibition of Foxp3 on RORγt can be removed and the development of Th17 be promoted[5,32]. Otherwise, Foxp3 promotes the development of Treg[33,34]. As we known, serum IL-6 level in SLE is increased[15], and IL-6 activates STAT3, thereby down-regulating the expression of Foxp3, resulting in the proportion of Th17 cells increases in SLE[13].

Our result showed that the proportion of CD4+IL-17A+(Th17) decreased significantly with the increasing BDNF concentration, while the proportion of CD4+CD25+CD127low(Treg) increased. In addition, we found that the expression of RORγtmRNA showed a decreasing intendancy, while the expression of Foxp3mRNA was increased. We proved that BDNF inhibited the differentiation of Th17 by down-regulating the
expression of RORytmRNA, and promoted the differentiation of Treg by up-regulating the expression of Foxp3mRNA. Previously, we have reported that serum BDNF level is lower in active stage of SLE[21]. We speculated that decreased BDNF level in serum of SLE probably weakened its inhibition on the proliferation of Th17, and the development of Th17 increased, participating in the pathogenesis of SLE. However, BDNF level gradually increased during the convalescence of the disease, restored its inhibition on Th17 and finally up-regulated the development of Treg. From the results above we speculate that BDNF appears to have a vital role in maintaining the balance of Treg/Th17 ratio.

Previous studies confirmed that the activity of PI3K/Akt pathway was enhanced in murine lupus[35]. The inhibitor of PI3K could improve the symptoms of glomerulonephritis in MRL/Faslpr SLE mice and reduced the mortality[36]. MAPK/ERK was involved in the pathogenesis of SLE[37] and MAPK inhibitor had been shown to reduce autoimmune responses[38]. The differentiation and function of Th17 are controlled by a variety of intracellular signaling pathways and complex transcription factor networks[39]. It was reported that the PI3K-Akt-mTORC1 axis had positive regulating effect on the differentiation of Th17[8,9,40]. In the CD4+T lineage, both PI3K and mTORC1 inhibitors can increase the differentiation of Treg[40]. Kurebayashi Y[39] also reported that the PI3K-Akt-mTORC1-S6K1 axis has positive regulating effect on the differentiation of Th17 by inhibiting the expression of Gfi1 and promoting nuclear translocation of RORyt[8]. As the downstream of mTORC1, S6K1 induces the expression of transcription factors EGR1 and EGR2[41], and EGR1 and EGR2 directly bind to the Gfi1 promoter to inhibit the expression of Gfi1 and accelerate the differentiation of Th17[8]. S6K2 is the nuclear counterpart of S6K1, which has the role of nuclear localization signal. S6K2 can transport RORyt to the nucleus after binding to RORyt by a back-loading manner. The expression of S6K2 is dependent partly on mTORC1 after TCR stimulation. Therefore, the PI3K-Akt-mTORC1-S6K2 pathway also up-regulates the differentiation of Th17 through the nuclear translocation of RORyt[8]. Otherwise, Th17 differentiation is also positively regulated by HIF-1, a transcription factor induced by hypoxia. Recent studies have shown that STAT3-induced HIF-1 binding to Foxp3 leads to the degradation of Foxp3 proteome[42], which removes its inhibition on RORyt. Reports have shown that both hypoxia and HIF-1 have positive and negative regulatory effects on the differentiation of Th7 and Treg respectively[43,44].

In addition to RORyt, STAT3 is also an important transcription factor for the differentiation of Th17[45], and IL-6 is the necessary factor for the activation of STAT3[46]. Ren[47] reviewed the mechanism that mTORC1 up-regulated the expression of IL-17 by STAT3, HIF-1, S6K1 and S6K2. In addition, as the downstream pathway of IL-6 and TGF-β, MAPK/ERK is also involved in differentiation of Th17 and the development of autoimmune diseases. Studies have shown that blocking the activation of ERK pathway can alleviate autoimmune response mediated by Th17 in EAE mouse model. Liu[10] reported the role of ERK in the development of Th17 and Treg. They demonstrated that blocking the activation of the IL-6-induced ERK pathway under the condition of Th17 polarization could down-regulate the expression of RORyt, inhibiting the differentiation of Th17, and up-regulate the differentiation of Treg. In vitro, they also demonstrated that T cells treated with ERK inhibitor produced more TGF-β, reduced differentiation of Th17, and reduced intestinal inflammatory response in colitis[10].
We explored the signal transduction pathway that BDNF affected differentiation of CD4+T. Our data showed that the phosphorylation of Akt, mTORC1 and ERK1/2 were increased after the addition of TGF-β+IL-6, and the proportion of CD4+IL-17A+ were increased, indicating that we successfully induced the differentiation of Th17 by activating the PI3K-Akt-mTORC1 axis and the ERK1/2 pathway. While the phosphorylation of Akt, mTORC1, ERK1/2 decreased in the BDNF group, the corresponding CD4+IL-17A+ ratio also declined. In the group pre-treated with TrkBlgG, there was no significant change in the phosphorylation of Akt and mTORC1. From the results above we hypothesized that combination of BDNF and TrkB may directly or indirectly down-regulate the development of Th17. We also proved that the PI3K-Akt-mTORC1 axis and ERK1/2 pathway were indeed affected by BDNF/TrkB complex. Similarly, Under the condition of Treg polarization induced by TGF-β, the phosphorylation of Akt and mTORC1 was also reduced in the BDNF group, nevertheless, the percentages of CD4+CD25+CD127low were increased. In the TrkBlgG group, there was no significant change in the phosphorylation of Akt and mTORC1. The phosphorylation of ERK1/2 was also decreased. Our data demonstrated that BDNF inhibited the development of Th17 regardless of Th17 or Treg polarization, and in turn promoted the development of Treg. Compared with initial CD4+T, under the condition of Th17 polarization, the activation of PI3K-Akt-mTORC1 axis was inhibited more obviously(Fig.3A). The amount of Th17 may be one of the reasons, or there may exist a certain correlation between BDNF and TGF-β or IL-6, or the downstream pathway of TGF-β and IL-6. Whether BDNF has a synergistic effect with TGF-β is indecisive. Although expressions of both Foxp3 and RORγt require TGF-β, the signaling cascade of downstream of TGF-β is different. For example, Smad4 seems to be necessary to induce both Foxp3 and RORγt, nevertheless, however, TGF-β induces Foxp3 expression through Smad2-/Smad3[48]. Smad pathway is necessary for TGF-β-induced Foxp3 expression[49]. It was reported that the ERK pathway negatively regulated the expression of Foxp3 and inhibited the differentiation of Treg, which is also dependent on the cytokine TGF-β[50]. In conclusion, the deep understanding about the molecular mechanism of BDNF affecting the PI3K- Akt - mTORC1 axis and ERK1/2 pathway remains to be further explored.

5. Conclusion

Our results demonstrated that BDNF was involved in the pathogenesis of SLE. BDNF not only promotes the proliferation of T lymphocytes, but also regulates the proliferation and differentiation of Treg and Th17. This discovery gives us a deeper understanding of the effect of BDNF on T lymphocytes, and BDNF may have profound and important meaning for autoimmune diseases.

Declarations

AUTHOR CONTRIBUTIONS

Bailing Tian and Xiaoyu Hou collect the samples and performed the experiments. Bailing Tian and Mengmeng Zhao collected and analyzed the data. Bailing Tian prepared the manuscript. Mengmeng Zhao contributed to the conception of the study. All authors have read and approved the manuscript.
ACKNOWLEDGMENTS

The author thanks the study participants. This work was supported by the following grants: foundation from Clinical Medical Research Center of Shenyang, Liaoning, China (18_009-4-03 to PT.Y.), foundation from the Major State Research Development Program of Liaoning, China (No. 2017225024 to PT.Y.), foundation from the Project for Construction of Major Discipline Platform in Universities of Liaoning province, China (2017001 to PT.Y.), the Program of the Distinguished Professor of Liaoning Province, Rheumatology (2017, Pingting Yang). None of the authors has any conflicts of interest to declare.

Availability of data and material:

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflicts of interest:

The authors declare that they have no competing interests.

Ethics approval:

The project was approved by the clinical scientific research section of the Ethics Committee of The First Hospital Of China Medical University (No. 2018-214-3).

Consent to participate:

This study was approved by the ethics committee of China Medical University (Shenyang, China). All procedures were following the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki.

Consent for publication:

All patients provided written informed consent.

Code availability:

Not applicable.

References
1. Sakaguchi. 2005 Apr. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and nonself. Nat Immunol. 6(4): 345-352.

2. Kleinewietfeld, and D.A. Hafler. 2013 Nov 15. The plasticity of human Treg and Th17 cells and its role in autoimmunity. Semin Immunol. 25(4): 305-312.

3. Qu, M. Xu, I. Mizoguchi, J. Furusawa, K. Kaneko, K. Watanabe, and J. Mizuguchi, et al. 2013 Jul 14. Pivotal roles of T-helper 17-related cytokines, IL-17, IL-22, and IL-23, in inflammatory diseases. Clin Dev Immunol. 2013:968549.

4. Zhu, and W.E. Paul. 2010 Nov. Peripheral CD4+ T-cell differentiation regulated by networks of cytokines and transcription factors. Immunal Rev. 238(1): 247-262.

5. Zhou, J.E. Lopes, M.M.W. Chong, I.L. Ivanov, R. Min, G.D. Victoria, and Y. Shen, et al. 2008 May 8. TGF-β-induced Foxp3 inhibits TH17 cell differentiation by antagonizing RORγt function. Nature. 453(7192): 236-240.

6. Bettelli, Y. Carrier, W. Gao, T. Korn, T.B. Strom, M. Oukka, and H.L. Weiner, et al. 2006 May 11. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature. 441(7090): 235-238.

7. Kawai, and S. Akira. 2011 May 27. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity. 34(5): 637-650.

8. Kurebayashi, S. Nagai, A. Ikejiri, M. Ohtani, K. Ichiyama, Y. Baba, and T. Yamada, et al. 2012 Apr 19. PI3K-Akt-mTORC1-S6K1/2 axis controls Th17 differentiation by regulating Gfi1expression and nuclear translocation of RORγt. Cell Rep. 1(4): 360-373.

9. Nagai, Y. Kurebayashi, and S. Koyasu. 2013 Mar. Role of PI3K/Akt and mTOR complexes in Th17 cell differentiation. Ann N Y Acad Sci. 1280:30-34.

10. Liu, S. Yao, S.M. Dann, H. Qin, C.O. Elson, and Y. Cong. 2013 Jul. ERK differentially regulatesTh17- and Treg-cell development and contributes to the pathogenesis of colitis. Eur J Immunol. 43(7): 1716-1726.

11. Bonelli, J.S. Smolen, and C. Scheinecker. 2010 Jan. Treg and lupus. Ann Rheum Dis. 69:Suppl 1: i65-66.

12. M. Talaat, S.F. Mohamed, I.H. Bassyouni, and A.A. Raouf. 2015 Apr. Th1/Th2/Th17/Treg cytokine imbalance in systemic lupus erythematosus (SLE) patients: Correlation with disease activity. Cytokine. 72(2): 146-153.

13. Valencia, C. Yarboro, G. Illei, and P.E. Lipsky. 2007 Feb 15. Deficient CD4+CD25high T regulatory cell function in patients with active systemic lupus erythematosus. J Immunol. 178(4): 2579-2588.

14. Li, B. Guo, H. Wu, L. Tan, C. Chang, and Q. Lu. 2015. Interleukin-17 in systemic lupus erythematosus: A comprehensive review. Autoimmunity. 48(6): 353-361.

15. Yoshio, H. Okamoto, K. Kurasawa, Y. Dei, S. Hirohata, and S. Minota. 2016 Aug. IL-6, IL-8, IP-10, MCP-1 and G-CSF are significantly increased in cerebrospinal fluid but not in sera of patients with central neuropsychiatric lupus erythematosus. Lupus. 25(9): 997-1003.
16. Zheng, X. Zhou, C. Moon, and H. Wang. 2012. Regulation of brain-derived neurotrophic factor expression in neurons. Int J Physiol Pathophysiol Pharmacol. 4(4): 188-200.

17. Kerschensteiner, E. Gallmeier, L. Behrens, V.V. Leal, T. Missgold, W.E. Klinkert, and R. Kolbeck, et al. 1999 Mar 1. Activated human T cells, B cells, and monocytes produce brain-derived neurotrophic factor in vitro and in inflammatory brain lesions: a neuroprotective role of inflammation? J Exp Med. 189(5): 865-870.

18. Nakahashi, H. Fujimura, C.A. Altar, J. Li, J. Kambayashi, N.N. Tandon, and B. Sun. 2000 Mar 24. Vascular endothelial cells synthesize and secrete brain-derived neurotrophic factor. FEBS Lett. 470(2): 113-117.

19. De Santi, L. Cantalupo, M. Tassi, D. Raspadori, C. Cioni, and P. Annunziata. 2009 Feb 15. Higher expression of BDNF receptor gp145trkB is associated with lower apoptosis intensity in T cell lines in multiple sclerosis. J Neurol Sci. 277(1-2): 65-70.

20. Garcia-Suarez, M.A. Blanco-Gelaz, and M.L. opez. 2002 Aug. Massive lymphocyte apoptosis in the thymus of functionally deficient TrkB mice. J Neuroimmunol. 129(1-2): 25-34.

21. Tian, C. Yang, J. Wang, X. Hou, S. Zhao, Y. Li, and P. Yang. 2019 Nov. Peripheral blood brain-derived neurotrophic factor level and tyrosine kinase B expression on T lymphocytes in systemic lupus erythematosus: Implications for systemic involvement. Cytokine. 123: 154764.

22. F. Tamashiro, R.D.R. Oliveira, R. Oliveira, E.R.C. Frota, E.A. Donadi, C.M. Del-Ben, and A.L. Teixeira, et al. 2014 Dec. Participation of the neutrophin brain-derived neurotrophic factor in neuropsychiatric systemic lupus erythematosus. Rheumatology. 53(12): 2182-2190.

23. Oku, T. Atsumi, Y. Akiyama, H. Amano, N. Azuma, T. Bohgaki, and Y.F. Asanuma, et al. 2018 Jul. Evaluation of the alternative classification criteria of systemic lupus erythematosus established by Systemic Lupus International Collaborating Clinics (SLICC). Mod Rheumatol. 28(4): 642-648.

24. D. Gladman, D. Ibanez, and M.B. Urowitz. 2002 Feb. Systemic lupus erythematosus disease activity index 2000. J Rheumatol. 29(2): 288-291.

25. A. Nockher, and H. Renz. 2003 Dec. Neurotrophins in inflammatory lung diseases: modulators of cell differentiation and neuroimmune interactions. Cytokine Growth Factor Rev. 14(6): 559-578.

26. A. Vega, O. García-Suárez, J. Hannestad, M. Pérez-Pérez, and A. Germanà. 2003 Jul. Neurotrophins and the immune system. J Anat. 203(1): 1-19.

27. Ikenouchi-Sugita, R. Yoshimura, N. Ueda, Y. Kodama, W. Umene-Nakano, and J. Nakamura. 2008 Dec. Continuous decrease in serum brain-derived neurotrophic factor (BDNF) levels in a neuropsychiatric syndrome of systemic lupus erythematosus patient with organic brain changes. Neuropsychiatr Dis Treat. 4(6): 1277-1281.

28. T. AbouGhanima, G.G. Elolemy, S.S. Ganeb, A.A. Abo Elazem, and E.R. Abdelgawad. 2012. Role of T helper 17 cells in the pathogenesis of systemic lupus erythematosus. Egypt J Immunol. 19(2): 25-33.

29. Zhou, B. Hu, N. Huang, X. Mo, W. Li, B. Zhang, and B. Wei, et al. 2018 Sep. Aberrant T cell subsets and cytokines expression profile in systemic lupus erythematosus. Clin Rheumatol. 37(9): 2405-2413.
30. Yu, M. Guan, Y. Peng, Y. Shao, C. Zhang, X. Yue, and J. Zhang, et al. 2011 Nov. Copy number variations of interleukin-17F, interleukin-21, and interleukin-22 are associated with systemic lupus erythematosus. Arthritis Rheum. 63(11): 3487-3492.

31. Manel, D. Unutmaz, and D.R. Littman. 2008 Jun. The differentiation of human TH-17 cells requires transforming growth factor-β and induction of the nuclear receptor RORyt. Nat Immunol. 9(6): 641-649.

32. Zhang, G. Meng, and W. Strober. 2008 Nov. Interactions among the transcription factors Runx1, RORgammat and Foxp3 regulate the differentiation of interleukin 17-producing T cells. Nat Immunol. 9(11): 1297-1306.

33. O. Yang, B.P. Pappu, R. Nurieva, A. Akimzhanov, H.S. Kang, Y. Chung, and L. Ma, et al. 2008 Jan. T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR alpha and ROR gamma. Immunity. 28(1): 29-39.

34. Park, Z. Li, X.O. Yang, S.H. Chang, R. Nurieva, Y.H. Wang, and Y. Wang, et al. 2005 Nov. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nature Immunol. 6(11): 1133-1141.

35. Niculescu, P. Nguyen, T. Niculescu, H. Rus, V. Rus, and C.S. Via. 2003 Apr. Pathogenic T cells in murine lupus exhibit spontaneous signaling activity through phosphate idylinositol 3-kinase and mitogen-activated protein kinase pathways. Arthritis and Rheumatism. 48(4): 1071-1079.

36. F. Barber, A. Bartolomé, C. Hernandez, J.M. Flores, C. Redondo, C. Fernandez-Arias, and M. Camps, et al. 2005 Sep. PI3Kγ inhibition blocks glomerulonephritis and extends lifespan in a mouse model of systemic lupus. Nature Medicine. 11(9): 933-935.

37. Bloch, M. Amit-Vazina, and E. Yona. 2014 Jun. Increased ERK and JNK activation and decreased ERK/JNK ratio are associated with long-term organ damage in patients with systemic lupus erythematosus. Rheumatology (Oxford). 53(6): 1034-1042.

38. Gorelik, and B. Richardson. 2010 Feb. Key role of ERK pathway signaling in lupus. Autoimmunity. 43(1): 17-22.

39. Kurebayashi, S. Nagai, A. Ikejiri, and S. Koyasu. 2013 Apr. Recent advances in understanding the molecular mechanisms of the development and function of Th17 cells. Genes Cells. 18(4): 247-265.

40. Sauer, L. Bruno, A. Hertweck, D. Finlay, M. Leleu, M. Spivakov, and Z.A. Knight, et al. 2008 Jun 3. T cell receptor signaling controls Foxp3 expression via PI3K, Akt, and Mtor. Proc Natl Acad Sci USA. 105(22): 7797-7802.

41. S. Carnevalli, K. Masuda, F. Frigerio, O.L. Bacquer, S.H. Um, V. Gandin, and I. Topisirovic, et al. 2010 May 18. S6K1 plays a critical role in early adipocyte differentiation. Dev Cell. 18(5): 763-774.

42. V. Dang, J. Barbi, H.Y. Yang, D. Jinasena, H. Yu, Y. Zheng, and Z. Bordman, et al. 2011 Sep 2. Control of TH17/Treg balance by hypoxia-inducible factor 1. Cell.146(5): 772-784.

43. Ikejiri, S. Nagai, N. Goda, Y. Kurebayashi, M. Osada-Oka, K. Takubo, and T. Suda, et al. 2012 Mar. Dynamic regulation of Th17 differentiation by oxygen concentrations. Int Immunol. 24(3): 137-146.
44. Z. Shi, R. Wang, G. Huang, P. Vogel, G. Neale, D.R. Green, and H. Chi. 2011 Jul 4. HIF1 alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. J Exp Med. 208(7): 1367-1376.

45. O. Yang, A.D. Panopoulos, R. Nurieva, S.H. Chang, D. Wang, S.S. Watowich, and C. Dong. 2007 Mar 30. STAT3 regulates cytokine-mediated generation of inflammatory helper T cells. J Biol Chem. 282(13): 9358-9363.

46. C. Heinrich, I. Behrmann, G. Müller-Newen, F. Schaper, and L. Graeve. 1998 Sep 1. Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. Biochem J. 334(Pt2): 297-314.

47. Ren, J. Yin, J. Duan, G. Liu, B. Tan, G. Yang, and G. Wu, et al. 2016 Feb. mTORC1 signaling and IL-17 expression: Defining pathways and possible therapeutic targets. Eur J Immunol. 46(2): 291-299.

48. Takimoto, Y. Wakabayashi, T. Sekiya, N. Inoue, R. Morita, K. Ichiyama, and R. Takahashi, et al. 2010 Jul 15. Smad2 and Smad3 are redundantly essential for the TGF-b-mediated regulation of regulatory T plasticity and Th1 development. J Immunol. 185(2): 842-855.

49. Derynck, and Y.E. Zhang. 2003 Oct 9. Smad-dependent and Smad-independent pathways in TGF-beta family signaling. Nature. 425(6958): 577-584.

50. Gabryšová, J.R. Christensen, X. Wu, A. Kissenpfennig, B. Malissen, and A. O’Garra. 2011 May. Integrated T-cell receptor and costimulatory signals determine TGF-beta-dependent differentiation and maintenance of Foxp3+ regulatory T cells. Eur J Immunol. 41(5): 1242-1248.