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Taurine corrects lupus CD4$^+$ T cell imbalance through inhibition of mTORC1 signaling

Saisai Huang[1†], Yiyuan Cui[2†], Yaqi Zhang[3], Hanyin Deng[3], Shanshan Liu[1*] and Xuebing Feng[1,3*]

Abstract

Objective This study was designed to explore the metabolism features in systemic lupus erythematosus (SLE) and to investigate the role and regulatory mechanism of taurine in the control of CD4$^+$T cells and the progression of SLE.

Methods Metabolomic profiles of sera from SLE patients and healthy controls (HCs) were analyzed by mass spectrometry. The therapeutic effects of taurine in vivo were observed in resiquimod (R848) induced mice, and the effects of taurine on various functions of CD4$^+$ T cells were examined by flow cytometry. The effect of mTORC1 agonist MHY1485 on the regulatory capacity of taurine was examined in vitro.

Results Both untargeted metabolomics assays and independent sample validation showed that serum levels of taurine were reduced in SLE patients compared to HCs (*P<0.0001*), which was inversely correlated with disease activity scores (*P<0.05*). Taurine supplementation relieved the progression of lupus in R848 induced mice, characterized by a decrease in anti-dsDNA (*P<0.01*) and proteinuria (*P<0.05*) and a reduction in the severity of nephritis (*P<0.05*). And, taurine supplementation improved the differentiation of cell subsets such as Th17 (*P<0.001*) and Treg cells (*P<0.001*) in these mice. In vitro, taurine suppressed reactive oxygen species production (*P<0.001*), proliferation (*P<0.0001*) and senescence (*P<0.0001*) of mouse spleen cells. The level of pS6 (*P<0.0001*) but not AKT in CD4$^+$ T was significantly decreased after taurine treatment, while mTORC1 agonists partially blocked the effect of taurine on CD4$^+$ T cells.

Conclusion Taurine may play a therapeutic role by ameliorating CD4$^+$ T cell abnormalities through inhibition of mTORC1 signaling in SLE.

Keywords Taurine, Metabolism, CD4$^+$ T cell, mTOR, Systemic lupus erythematosus

Introduction

Systemic lupus erythematosus (SLE) is a clinically heterogeneous disease characterized by disruption of immune tolerance, leading to activation of both innate and adaptive immune responses against nucleic acids and endogenous antigens, which cause damage to various body systems [1]. T cells play a vital role in the pathogenesis of SLE through the secretion of pro-inflammatory cytokines to amplify the inflammatory response, and to provide assistance to B cells, leading to aberrant immune cell function and tissue damage [2]. In recent years, data are increasingly emerging on how metabolic processes control the function of various T cell subsets and how these

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metabolic processes are altered in SLE [3, 4]. Notably, metabolic characteristics of SLE T cells, such as enhanced glycolysis and lipid synthesis, have been reported [5, 6], yet it remains unclear how specific metabolites regulate SLE T cell function, especially $CD4^+$ T cells.

Taurine is a conditionally essential micronutrient and an abundant amino sulfonic acid that has pleiotropic cellular and physiological functions, particularly in the context of metabolic homeostasis [7]. Taurine may improve cognition function by attenuating neuroinflammarion and antioxidant capacities [8], and its deficiency is thought to be a driver of aging [9], whereas N-acetyltaurine, a secondary metabolite of taurine, regulates feeding and obesity through the orphan enzyme phosphotriesterase-related (PTER) [10]. Taurine metabolism is involved in the regulation of lupus autoimmunity, but current reports are highly contradictory. For example, one study has shown that taurine inhibits M1 polarization and promotes M2 polarization in macrophages, thereby alleviating lupus nephritis [11], while another study proposed that taurine exacerbates the development of lupus by promoting the function of plasmacytoid dendritic cells [12].

Toll-like receptor 7 (TLR7) is a pattern recognition receptor that senses single-stranded RNA (ssRNA) from viruses and host cells. Aberrations in TLR7 are sufficient to trigger lupus, which has recently been demonstrated in humans [13]. Consequently, induction of systemic autoimmunity in wild-type mice by epicutaneous administration of TLR7 agonists has emerged as a new approach to modeling SLE [14]. In addition, mammalian target of rapamycin (mTOR) is a central regulator of cellular metabolism and plays a key role of conventional T lymphocytes proliferation and development [15]. Accordingly, taurine, an amino acid metabolite, was screened by serum metabolomics in SLE patients, taking into account both the function of the metabolite and its association with the disease, and applied to treat TLR7 agonist R848 induced lupus mouse model for the elucidation of its effect on mTOR regulation in $CD4^+$ T cells. To the best of our knowledge, this is the first study to systematically address the regulation of taurine on lupus $CD4^+$ T cell function.

Materials and methods

Patients and controls

Study protocol was reviewed and approved by the Ethics Committee at The Affiliated Drum Tower Hospital of Nanjing University Medical School (2021-035), and all participants provided written informed consents. To perform untargeted metabolic analysis, 30 patients who fulfilled the 1997 American College of Rheumatology (ACR) diagnostic criteria for SLE [16] and 30 HCs matched for age and gender were recruited. To validate the results, another cohort of 40 SLE patients and 18 HCs were further recruited. Excluded criteria were (1) infection, malignancy and pregnancy lactation; (2) presence of comorbid autoimmune diseases; (3) presence of comorbid metabolic diseases (e.g., hyperthyroidism, hyperemia, hypertension, diabetes, coronary heart disease, or other organic diseases).

Patient clinical profiles were collected from medical records through an electronic data system and present in Table 1. The data collected included demographic information, blood routine (white blood cells [WBC], hemoglobin [HB], platelet [PLT]), kidney function (24 h-albuminuria, endogenous creatinine clearance rate [eGFR]), immunity system (complement component 3 [C3], complement component 4 [C4], Immunoglobulin G [IgG], Immunoglobulin M [IgM]), systemic inflammation (C-reactive protein [CRP], erythrocyte sedimentation rate [ESR], prednisone-equivalent glucocorticoids [GCs]). Disease activity was defined according to systemic lupus erythematosus disease activity index (SLEDAI) [17].

Mice

The animal use and the experimental protocols of this study were approved by the Ethics Committee at The Affiliated Drum Tower Hospital of Nanjing University Medical School (2021AE01023). Female C57BL/6J mice were purchased from SPF Biotechnology Co., Ltd. (Beijing, China). Mice were housed in a specific pathogen-free environment and were housed in a temperature controlled room (21 to 26℃) with a 12-hour alternating light/dark cycle. For resiquimod (R848) (Sigma-Aldrich, USA)-treated mice models, 6-week-old female C57BL/6J mice were randomly assigned to R848 or acetone control groups as described previously [14]. The treatment of taurine in R848 groups was performed on the onset of disease (that was synchronized R848). Briefly, the skin of C57BL/6J mice on the right ear was treated topically, three times weekly, with 80ug of R848 in 40ul of acetone or 40ul of acetone alone for 7 weeks, and then sacrificed at 13 weeks. At the same time, mice were treated with 200μL sterile pyrogen-free physiological saline containing taurine (Sigma-Aldrich, USA) (150 mg/kg) or an equal volume of physiological saline via intraperitoneal injection every day for 7 consecutive weeks. The weight of these mice was evaluated every week, the blood samples, spleen and kidney were collected. When mice were sacrificed, the spleen index (ratio of spleen weight to body weight) were calculated.

Untargeted metabolic analysis

Blood samples were collected from the patients and HCs, and serum was extracted from the supernatant, frozen and stored at −80℃. The compounds in serum

Table 1 Clinical and demographic characteristics of SLE and HC groups

	Discovery cohort		Validation cohort	
Variable	**SLE**	**HC**	**SLE**	**HC**
Case number	30	30	40	18
Age (years) [a]	33.9 ± 10.1	31.2 ± 4.5	35.9 ± 10.5	32.2 ± 10.3
Disease duration, yrs [a]	6.3 ± 5.6		5.7 ± 5.2	
Gender (F/M)	29/1	30/0	35/5	16/2
SLEDAI [a]	18.9 ± 8.5	-	17.5 ± 4.8	
GC dose (mg/d) [a]	27.9 ± 26.6		33.6 ± 36.6	
Organ involvement (n,%)				
Renal involvement	24,80%		31,77.50%	
Hematologic	11,36.67%		15,37.50%	
Neuropsychiatric	2,6.67%		3,7.50%	
Musculoskeletal	12,40.00%		16,40.00%	
Cutaneous	16,53.33%		18,45.00%	
Gastrointestinal	2,6.67%		3,7.5%	
Laboratory data				
C3 (mg/dl) [a]	0.7 ± 0.3		0.7 ± 0.3	
C4 (mg/dl) [a]	0.1 ± 0.05		0.1 ± 0.08	
ESR (mm/h) [a]	40.6 ± 24.9		40.9 ± 32.7	
CRP (mg/l) [a]	15.93 ± 35.7		7.6 ± 10.9	
Cr (mg/dl) [a]	91.4 ± 98.2		109.9 ± 133.3	
BUN (mg/dl) [a]	8.5 ± 5.4		10.1 ± 6.2	
eGFR(m/min/1.73m^2) [a]	112.6 ± 56.3		101.6 ± 58.3	
Proteinuria (mg/24hr) [a]	2734.2 ± 4530.4		5174 ± 6144	
Treatment (n,%)				
Glucocorticoid	29, 96.7%		40, 100%	
Cyclophosphamide	10, 33.3%		13, 32.5%	
Hydroxychloroquine	19, 63.3%		28, 70%	
Mycophenolate mofetil	7, 23.3%		10, 25%	
Cyclosporin A	4, 13.3%		6, 15%	
Tacrolimus	7, 23.3%		8, 20%	

[a]Mean ± standard deviation

SLEDAI, systemic lupus erythematosus disease activity index; GC, Prednisone-equivalent glucocorticoid; C3,complement component 3; C4, complement component 4; ESR, erythrocyte sedimentation rate; Cr, creatinine; BUN, blood urea nitrogen; eGFR, endogenous creatinine clearance rate

were separated using an Agilent 1290 Infinity LC UHPIL HILIC during the analyses which was performed as previously described [18].

Original metabolomic data were handled with peak alignment, retention time adjustment, and extraction of the peak area for XCMS analysis. Ion peak data handled with XCMS were excluded when the total of groups exceeded 66.67%. The remaining data were normalized using Paretoscaling, and the valid data were handled with multivariate data analysis through MetaboAnalyst ((https://www.metaboanalyst.ca/), including principal component analysis (PCA) and supervised partial least square-discriminate analysis (PLS-DA). A supervised orthogonal projection to latent structures-discriminant analysis (OPLS-DA) was implemented using the EZinfo Software 2.0 (UpdateStar, Berlin, Germany). The candidate bines (i.e., chemical shifts) were identified from the scores of variable importance on projection (VIPs > 1) in OPLS-DA models. We performed a test with 200 permutations to assess the validity of the discriminant models to avoid overfitting. The valid data were handled with unidimensional statistical analysis through R software, such as the t-test, variation ratio analysis, and volcano plot analysis. Metabolites with VIPs > 1 and $P<0.05$ were used for pathway enrichment analysis in Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/) and MetaboAnalyst database (https://www.metaboanalyst.ca/). The Spearman's correlation was used to describe the relationships between different metabolites.

LC-MS/MS

The concentration of taurine in serum was analyzed using liquid chromatography (LC), taurine standards and internal standards were purchased from China Pharmaceutical and Biological Products Control Institute. Methanol and acetonitrile (MS grade) were obtained from Fisher Scientific (USA), and formic acid (MS grade) was sourced from Merck (USA). Water used in the experiment was prepared using a PureLab Classic UVF system (ELGA LabWater, UK). Chromatographic separation was performed using an Agilent InfinityLab Poroshell 120 EC-C18 column (2.1 × 150 mm, 2.7 μm) at a flow rate of 0.4 mL/min. Mass spectrometry was conducted on a triple quadrupole system (Qlife Lab 9000plus, Nanjing, China) operating in positive electrospray ionization (ESI+) mode.

Flow cytometry analysis

Antibodies used for cell staining were listed in Table S1. Data were acquired using a flow cytometer (BD LSR Fortessa). For the staining of surface antigens, cells were incubated with FITC-, PE-, PE-Cy7-, PE-CY7-, BV421-, APCCY7-, BV605-, or APC-conjugated monoclonal antibodies or their negative control antibodies as indicated for 30 min on ice. For the staining of intracellular cytokines, cells were pretreated with phorbol 12-myristate 13-acetate, ionomycin, and brefeldin A in RPMI1640 medium containing 10% fetal bovine serum (FBS; complete medium) for four hours, intracellular staining of Foxp3, IFNγ, IL-4, IL-17 A, P21 was performed. For intranuclear Ki67 staining, cells were fixed using eBio fix/perm kit (eBioscience, California, USA), washed

and stained with PE-CY7 Ki67 antibody in 1× eBio fix/perm buffer for one hour at 4 ℃. To detect cell apoptosis, Annexin V-PE/7-AAD Apoptosis kit (MultiSciences, Hangzhou, China) was used. To detect cell senescence, Cellular Senescence Detection Kit–SPiDER-βGal (DOJINDO, Japan) was used. For detection of phosphorylated signaling proteins p-S6 and p-AKT, mouse splenocytes were rested in complete medium for 24 h with anti-CD3 and anti-CD28 monoclonal antibody (mAb; eBioscience, California, USA). Subsequently, cells were surface stained, fixed, and then stained with antibodies to phosphorylated signal proteins.

Cell isolation and co-culture

The peripheral blood mononuclear cells (PBMCs) of SLE patients was isolated by density gradient centrifugation, and cultured with different dose of taurine, in the presence of 1 μg/ml anti-CD3/CD28 antibodies (Biolegend, San Diego, CA, USA). For the concentration gradient study, taurine was added at 2, 20, and 50mM. After 24 h, PBMCs were harvested, the percentage of Treg and Th17 were detected by flow cytometry. Spleens were dissected from mice and mononuclear cell suspensions were prepared after red blood cells were lysed. Cells were cocultured in the presence or absence of 20 mM taurine (Sigma, St. Louis, MO, USA), in the presence of 1 μg/ml anti-CD3/CD28 antibodies (Biolegend, San Diego, CA, USA). For rapamycin activation experiment, splenocytes were cultured with or without MHY1485 (5 μm) (MedChemexpress, New Jersey, USA) for 24 h.

Renal histology and immunofluorescence

When mice were sacrificed, kidneys were collected. One kidney was fixed in 4% paraformaldehyde (PFA), embedded in paraffin, sectioned at 3 μm, stained with hematoxylin and eosin (H&E; Sinopharm Chemical Reagent, Shanghai, P.R. China) according to standard procedures. The other one kidney was snap frozen in liquid nitrogen and placed in optimal cutting temperature (OCT) embedding matrix (Leica Biosystems, Nussloch, Germany). Immunofluorescent staining with fluorescein-conjugated anti-mouse IgG and C3 antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was performed on frozen kidney Sect. (3 μm) according to standard procedures. Glomerular C3 and IgG staining was graded according to intensity on a scale of 0 to 3 as follows: 0, absent; 1, faint; 2, intense; 3, very intense.

ELISA

Serum levels of IgG, Anti-double stranded (ds) DNA were determined with mouse IgG ELISA Kit (MultiSciences and AiFang biological, Hangzhou, China), mouse anti-dsDNA ELISA kit (Shibayagi, Gunma, Japan), respectively, according to the manufacturer's instructions. Urine protein, blood urea nitrogen (BUN) were measured using Urine protein test kit, Urea Assay Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to standard procedures.

Statistical analysis

The data analysis was performed using Graphpad prism 8.5 software. Continuous data were expressed as the mean ± standard deviation (SD). The Shapiro-Wilk test was applied to test whether the data were normally distributed. Mann-Whitney U test and 2-tailed unpaired t-test were used for comparison of two groups. One-way ANVOA with Tukey's method was used for comparisons of more than two groups. Both Spearman and Pearson correlation coefficients were used for correlation analyses. $P < 0.05$ were considered to be significant, and the changes of metabolites between SLE patients and HCs were determined with statistical significance established at a threshold of Bonferroni-adjusted $P \leq 0.0005$.

Result

Differential serum metabolites in SLE patients

To explore the abnormal metabolites in lupus, we systematically analyzed the changes of metabolites between 30 SLE patients and 30 HCs by using ultra-high-pressure liquid chromatography and quadrupole-time-of-flight mass spectrometry. The median disease duration of SLE patients was 6.3 years, and the median SLEDAI score was 17. In the plot results of OPLS-DA model, SLE patients and HCs were separated into two different clusters in metabolomics [R2Y (cum) = 0.981, Q2 (cum) = 0.857] (Fig. 1A), and the permutation test showed that the model was well stabilized (Fig. 1B).

The volcano plot displayed the distribution of differentially expressed metabolites (Fig. 1C), with 98 different metabolites between SLE patients and HCs, of which 45 were increased and 53 decreased in patients, as shown in Fig. 1D (supplementary Table 2 listed the metabolites significantly increased or decreased). After Bonferroni-adjusted, there were 48 different metabolites between SLE patients and HCs (supplementary Table 2). The top three categories of differential metabolites were lipid and lipid-like molecules (34.03%), organic acids and their derivatives (21.00%), and organic heterocyclic compounds (10.44%) (Fig. 1E). As one of the key molecules of organic acids and derivatives, taurine displayed significantly difference between SLE patients and HCs (Fig. 1F).

Reduced serum taurine levels in independent samples of SLE patients

To verify the results from untargeted metabolomics, we assembled another inpatients cohort of 40 lupus patients and 18 HCs, and measured serum taurine levels by LC-MS/MS. Our data showed that serum taurine

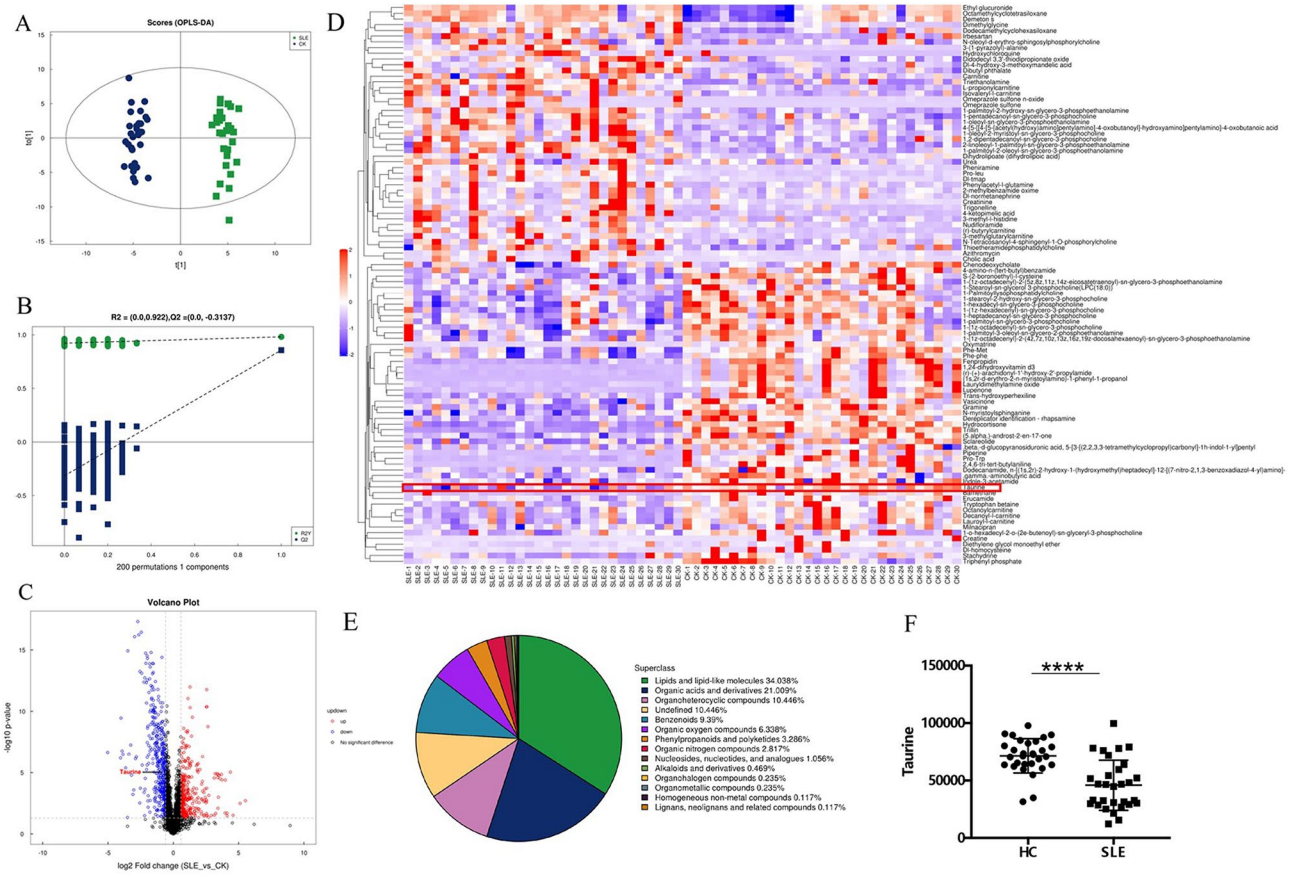

Fig. 1 Differential serum metabolites in SLE patients. (**A-B**) The supervised orthogonal partial least squares-discriminant analysis (OPLS-DA) score plot and 200 permutations of OPLS-DA between SLE patients and HCs. (**C**) Inter-group volcano identified several metabolites between SLE and HC groups. (**D**) Hierarchical clustering revealed significantly and differently expressed metabolites. (**E**) Classified pie chart of HMDB superclass. The numbers and proportions of differential metabolites are shown. (**F**) The concentration of Taurine was significantly lower in SLE patients than HCs. ****$P<0.0001$

levels were 91.12 ± 34.78 μM, which was lower than that in HCs ($p < 0.05$) (Fig. 2A) and negatively associated with SLEDAI scores (r=-0.38, $p = 0.01$) (Fig. 2B). Interestingly, serum taurine levels were positively correlated with the levels of WBC, HB and PLT, as well as complement C3 and C4 (Fig. 2C-F, H). Taurine levels were weakly and negatively correlated with IgG (Fig. 2G), whereas there was no correlation with ESR or proteinuria or GC (Fig. 2I-K). The effect of taurine on T cell subsets of SLE patients were checked in vitro, showing that taurine addition increased Treg cells and decreased Th17 cells in a dose-dependent manner (Fig. 2L and M), suggesting that taurine was involved in the regulation of $CD4^+$ T cells. Our data showed that the regulation of Treg and Th17 cells by taurine in SLE patients seemed not to be relevant to their disease activity (Fig S1A-B). Interestingly, taurine addition did not change the ratio of Treg cells and Th17 cells in unstimulated or stimulated PBMCs of HCs (Fig S1C-F), indicating that taurine supplementation may only act in the disease states.

Taurine ameliorated lupus symptoms in R848 induced mice

Compared to acetone-treated B6 mice, R848 induced mice displayed lupus-like manifestations, including splenomegaly, increased levels of proteinuria, serum creatinine, total IgG and anti-dsDNA antibody, as well as increased deposition of IgG and C3 and infiltrated leukocytes in kidney sections (Fig. 3). After taurine treatment, all R848-induced mice were kept alive (Fig. 3A), and their body weight increased (Fig. 3B). Compared with R848 induced mice, there was significantly lower spleen index, IgG, anti-dsDNA antibody, proteinuria and urine BUN after taurine treatment (Fig. 3C-E). In addition, taurine treatment markedly improved renal pathology in R848 induced mice, with less renal interstitial and perivascular lesions, decreased deposition of C3 and IgG in glomeruli (Fig. 3F-G), along with decreased levels of ROS in kidney (Fig. 3H). These findings confirmed that taurine supplement helped alleviate lupus symptoms.

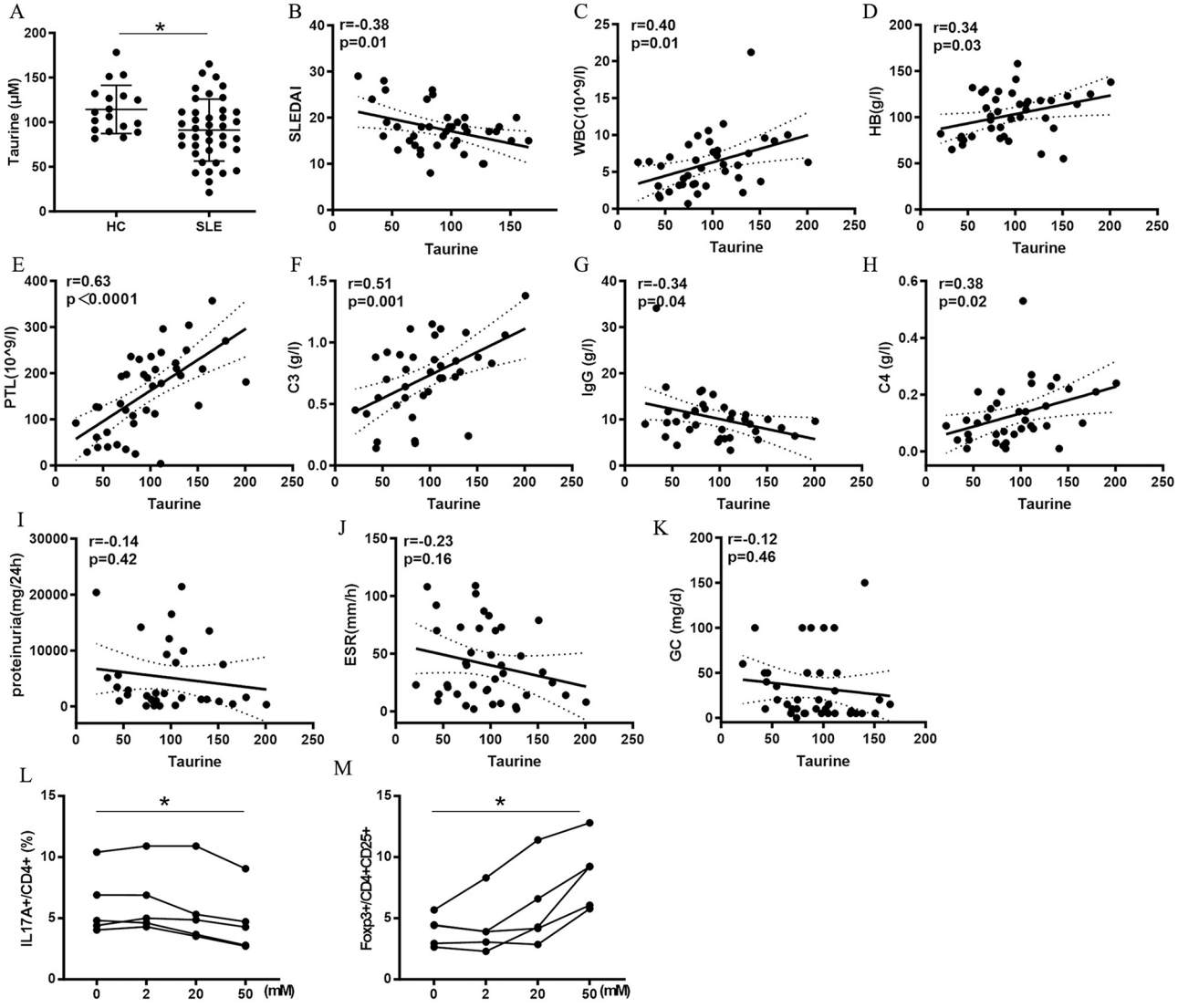

Fig. 2 Confirmation of reduced serum taurine levels in independent samples of SLE patients. (**A**) Decreased serum level of taurine in SLE patients (95% CI 4.66 to 41.76 μm) compared to that in healthy controls. (**B-K**) Correlation of taurine levels in SLE serum with the SLE Disease Activity Index (SLEDAI) (95% CI -0.62 to -0.078), white blood cells (WBC) (95% CI 0.11 to 0.64 10^9/l), hemoglobin (HB) (95% CI 0.027 to 0.59 g/l), platelet (PLT) (95% CI 0.39 to 0.79 10^9/l), complement component 3 (C3) (95% CI 0.23 to 0.72 g/l), complement component 4 (C4) (95% CI 0.058 to 0.62 g/l), Immunoglobulin G (IgG) (95% CI -0.60 to -0.015 g/l), erythrocyte sedimentation rate (ESR) (95% CI -0.50 to 0.091 mm/h), 24 h-albuminuria(95% CI -0.46 to 0.21 mg/24 h), GC (95% CI -0.42 to 0.19). (**L-M**) The PBMCs of SLE patients were cultured with different dose of taurine, in the presence of anti-CD3/CD28 antibodies for 24 h, the percentage of T helper 17 (Th17) cells and regulator T (Treg) cells were detected by flow cytometry. *$p < 0.05$

Taurine helped restore CD4$^+$ T cell balance

Compared to control B6 mice, R848 induced mice displayed increased percentages of Th1, Th17 and Tfh cells, but decreased percentages of Th2 and Treg cells in their splenic CD4$^+$ cells (Fig. 4A-E). Taurine treatment down-regulated the proportion of Th1 and Th17 cells (Fig. 4A-B), up-regulated Treg cells and Th2 cells in spleen (Fig. 4C-D), but had no significant effect on the frequency of Tfh cells (Fig. 4E).

To confirm the effect of taurine on CD4$^+$ T cells, splenic cells from control B6 mice or R848 induced mice were cultured with or without taurine in the presence of anti-CD3 and anti-CD28 for 24 h. Our data showed that ROS production, proliferation (Ki-67$^+$), activation (CD69$^+$), apoptosis (Annexin V$^+$ 7AAD$^+$) and senescence (SPIDER-βGal$^+$ and P21$^+$) were all increased in CD4$^+$ T cells of R848 induced mice (Fig. 4F-K). The addition of taurine rescued the abnormal oxidation, proliferation and senescence of CD4$^+$ T cells, while there was no significantly difference in cell activation and apoptosis. Collectively, these evidences revealed that taurine could partially correct the immune imbalance of CD4$^+$ T cells.

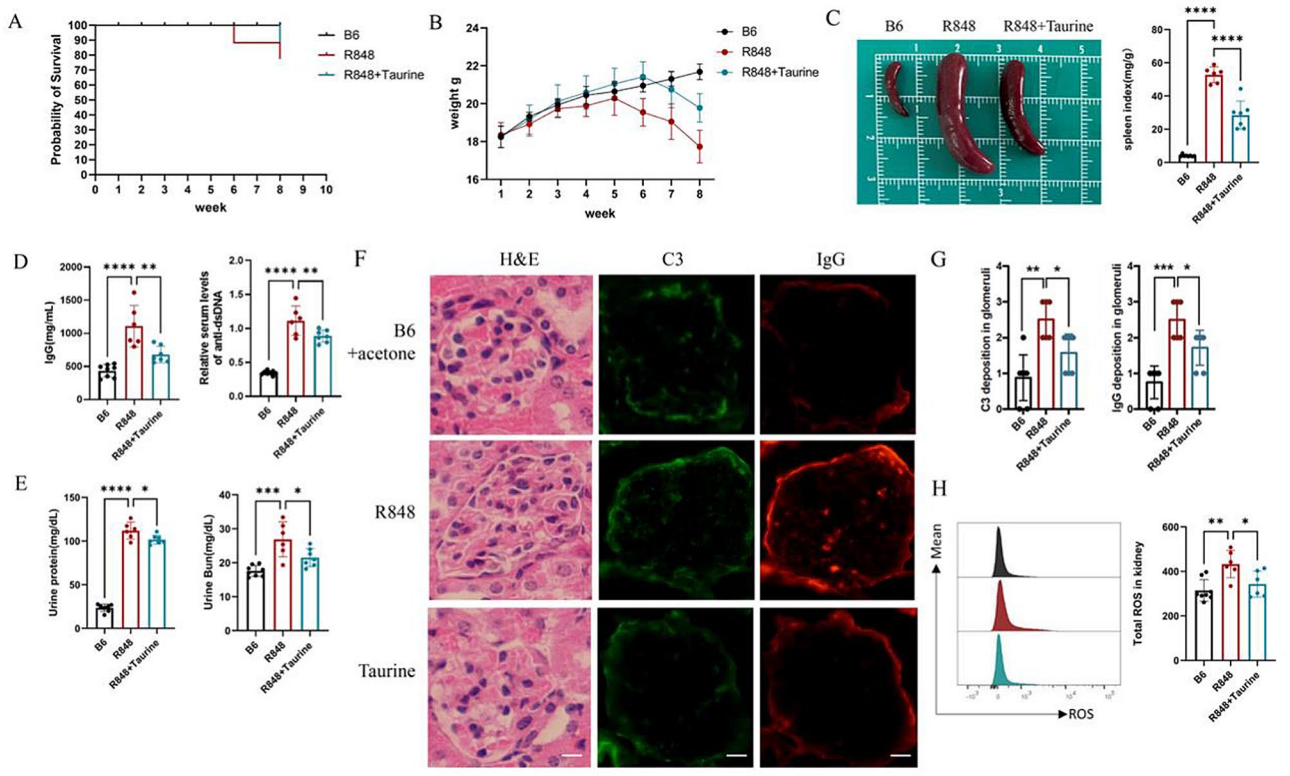

Fig. 3 Taurine ameliorated the progression of lupus symptoms in R848 lupus mice. (**A**-**B**) The survival curves and body weight of acetone-treated control B6 mice, R848-treated B6 mice and Taurine-treated R848 induced B6 mice. (**C**) Images of spleens and quantification of spleen weights among three groups of mice. (**D**)The levels of serum total IgG and anti-double stranded (ds)DNA antibody among different groups. (**E**). The levels of urine protein and blood urea nitrogen (BUN) of the three groups. (**F**) Representative images of kidney Hematoxylin and eosin staining (H&E) Sect. (400×magnification), C3 and IgG immunostaining (400× magnification). (**G**) The immune complex deposition scoring. (**H**) The frequency of ROS in kidney of the three groups of mice. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$

Taurine regulated CD4$^+$ T cells through the mTORC1 pathway

Our next question was how does taurine regulate CD4$^+$ T cells in lupus? As the mTOR pathway is closely linked to intracellular metabolism, phosphorylation levels of ribosomal protein S6 and protein kinase AKT were assessed by flow cytometry. Interestingly, both CD4$^+$ and CD8$^+$ T cells of R848 induced mice displayed enhanced phosphorylation-S6 and AKT, yet taurine treatment inhibited only the production of phosphorylation-S6 and had little effect on AKT in CD4$^+$ T cells, suggesting that it may act primarily through the regulation of mTORC1 in CD4$^+$ T cells (Fig. 5A). Consistently, the addition of the mTORC1 inhibitor MHY1485 to the cultures blocked the functional changes in CD4$^+$ T cells induced by taurine, despite having no effect on CD4$^+$ T cell numbers (Fig. 5B). MHY1485 counteracted the inhibitory effect of taurine on ROS generation and led to an increase in Ki-67, SPiDER-βGal, P21 and a decrease in Treg differentiation (Fig. 5C), supporting that mTORC1 is a pivotal pathway for taurine in the regulation of CD4$^+$ T cells.

Discussion

In this study, differential metabolites were detected and analyzed in the serum of SLE patients. Our findings clearly demonstrate that taurine levels were reduced in SLE patients and that the addition of taurine improved the conditions of lupus mice, characterized by decreased autoantibodies and proteinuria, less severe nephritis. Mechanistically, taurine helped to improve T-cell function, in particular reducing the production of ROS, inhibiting cell proliferation and ameliorating senescence, and restoring the proportions of its major subsets. This effect was achieved partially by counteracting the mTORC1 pathway.

SLE metabolism have increasingly attracted the attention of rheumatologists mainly because many metabolites expand in SLE patients correlating with disease activity [19–21]. Metabolic pathways can regulate T cell differentiation and function, thus contributing to SLE inflammation [22]. Several clinical studies investigating the therapeutic potential of taurine have been reported, such as cardiovascular disease, metabolic syndrome, neurological disorders, tumor and aging [23–25].

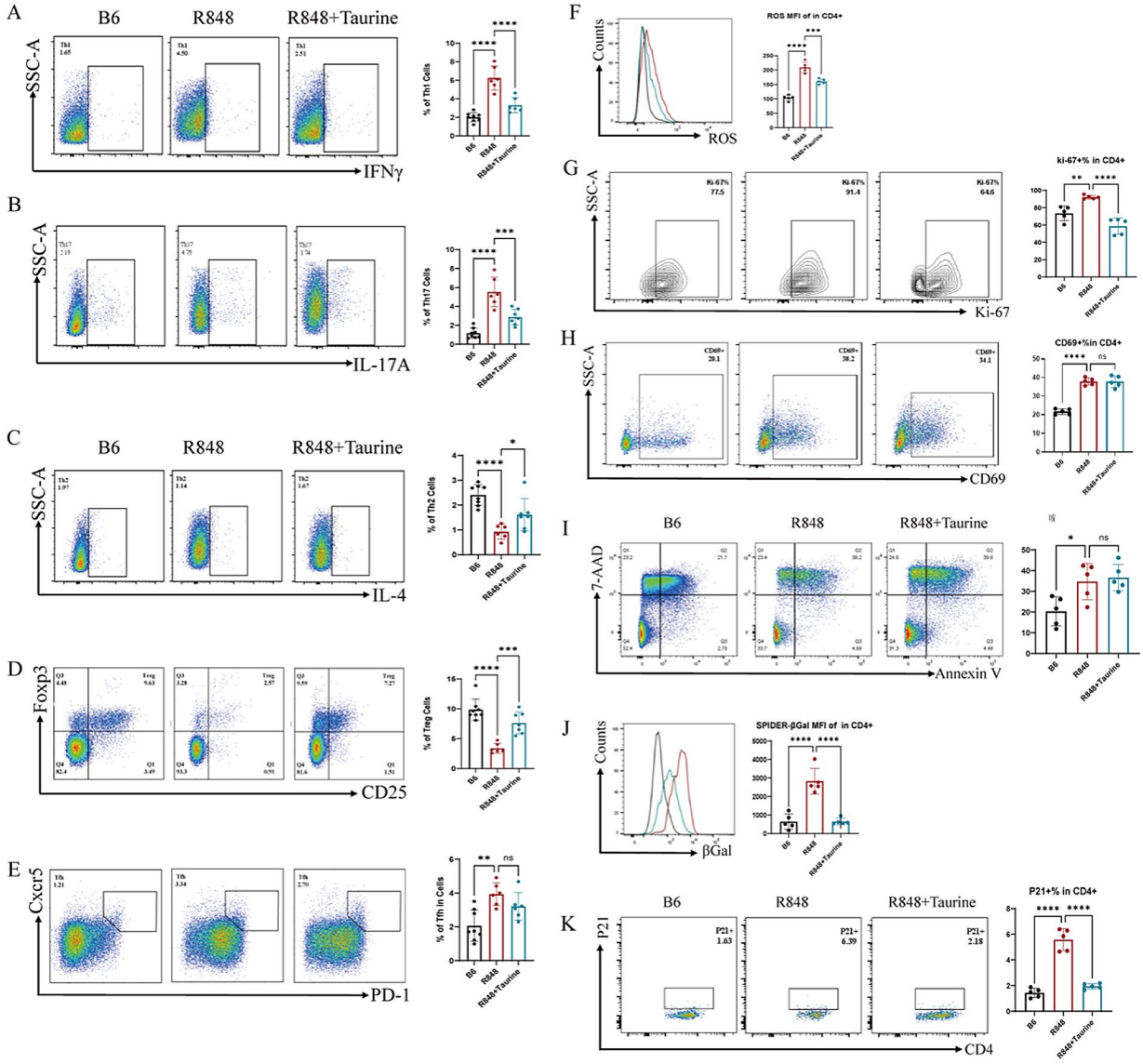

Fig. 4 Taurine helped restore CD4$^+$ T cell balance. (**A**-**E**) Splenocytes from acetone-treated control B6 mice, R848-treated B6 mice and taurine treated R848 lupus mice, the percentages of T helper 1 (Th1), T helper 17 (Th17), T helper 2 (Th2), regulator T (Treg), T follicular helper (Tfh) cells. (**F**-**K**) Splenocytes from acetone-treated control B6 mice, R848-treated B6 mice were stimulated with anti-CD3/CD28 antibodies and taurine for 24 h, comparison of the proportion of ROS (ROS production), Ki-67 (cell proliferation), CD69 (cell activation), Annexin V$^+$ 7AAD$^+$ (cell apoptosis), SPiDER-βGal and P21 cells (cell senescence) in splenic CD4$^+$ T cells of the three groups. $p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$

However, previous studies on taurine in SLE were controversial. In one study, serum taurine levels were found to be comparable in SLE patients and HC, but were significantly higher in those with lupus nephritis [26]. In another study, serum levels of taurine were elevated in SLE patients and were involved in the pathogenesis of SLE by enhancing plasma cell dendritic cell-mediated production of type I IFN [12]. Alternatively, it has been reported that patients with juvenile SLE presented significantly reduced levels of taurine [27], and taurine could attenuate renal damage and adjust the immune response in MRL/lpr mice [28]. There is also no clear conclusion of taurine function on inflammation. Some studies reported that taurine alleviates inflammation induced by Streptococcus through inhibiting macrophages M1 polarization. However, others suggest opposite [29–31].This discrepancy may due to the number, race, ethnicity and subtype of patients and animal model enrolled in.

MTOR is a master regulator of cell growth and metabolism, mTOR complex 1 (mTORC1) and mTORC2 are two distinct structurally and functionally complexes of mTOR [32]. MTOR signaling, is a core target for immunometabolism and could integrate metabolic signals with immune signals to direct the proper maintenance and

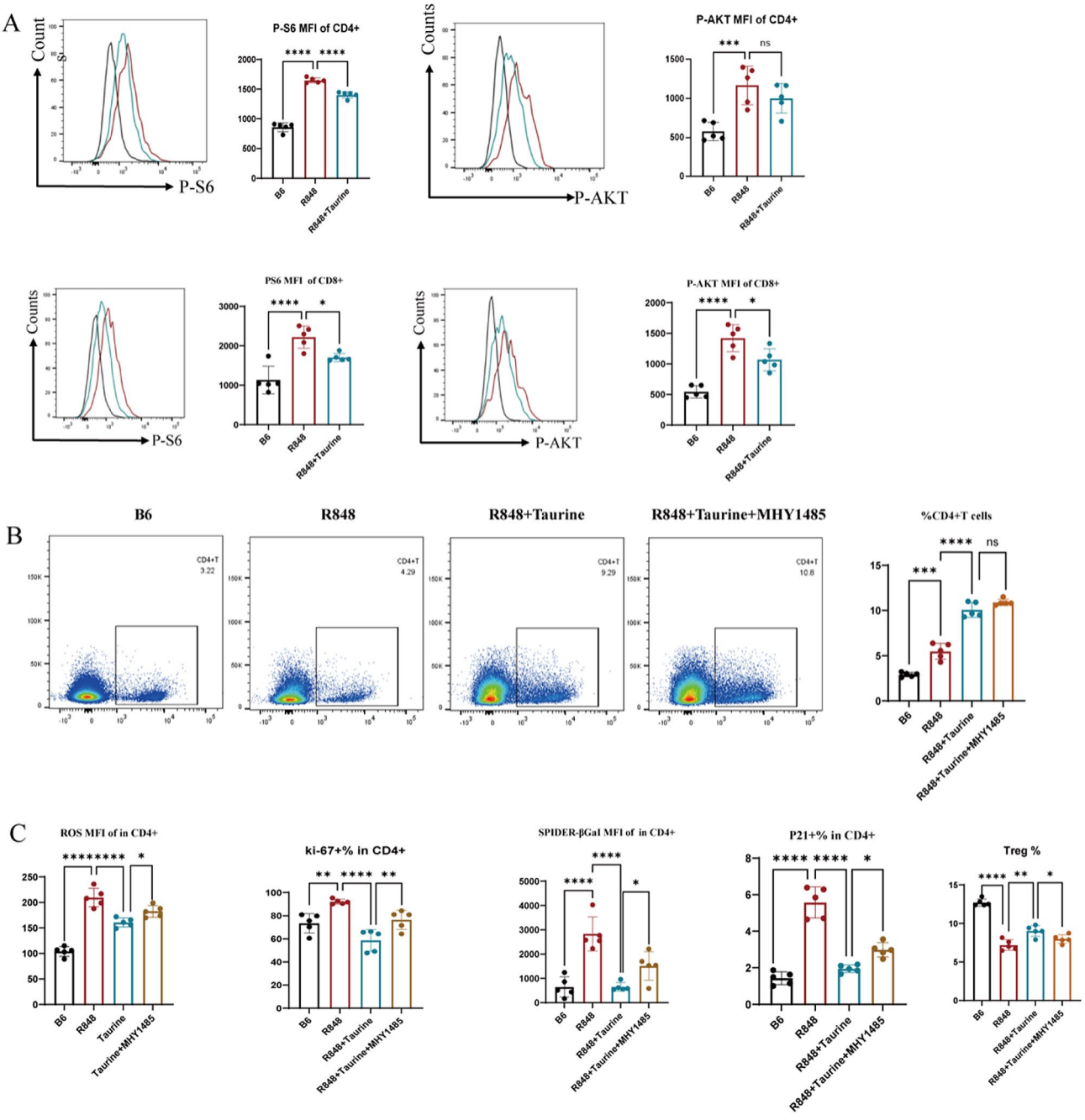

Fig. 5 Taurine regulated CD4$^+$ T cells through the mTORC1 pathway. (**A**) Splenocytes from acetone-treated control B6 mice, R848-treated B6 mice were stimulated with anti-CD3/CD28 antibodies and taurine for 24 h, then analyzed for phosphorylation of indicated proteins. Graphs show mean fluorescence intensities of p-S6, p-AKT in CD4$^+$ and CD8$^+$ T cells. (**B**) The percentages of CD4$^+$ T cells of acetone-treated control B6 mice, R848-treated B6 mice cultured with taurine and with or without MHY1485 for 24 h. (**C**) The percentages of ROS, Ki-67, SPiDER-βGal, P21 and Treg in CD4$^+$ T cells of of acetone-treated control B6 mice, R848-treated B6 mice cultured with taurine and with or without MHY1485 for 24 h. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$

activation of T cells [33, 34]. It has been reported the metabolic characteristics of SLE T cells contain highly activated mTOR [35], many clinical trials showed that sirolimus, an mTOR inhibitor, could alleviate the disease activity of SLE patients [36, 37]. Consistently, our previous work had found that phosphatidic acid promoted the generation of IL-17 A producing double-negative T cell by enhancing mTORC1 signaling in lupus [38]. In this study, we revealed for the first time that CD4$^+$ T cells abnormalities in SLE were partially regulated by taurine-dependent mTORC1 pathway. Amino acid metabolites, as represented by taurine, may inhibit mTORC1 signaling to modulate CD4$^+$ T cells oxidant, and then reduce

the proliferation, senescence and differentiation of $CD4^+$ T cells.

The pathogenesis of SLE is linked to the dysregulation of diverse immune cell populations, particularly $CD4^+$ T cell, DC and macrophage. In our study, we found taurine can modulate the function of $CD4^+$ T-helper lymphocytes. Notably, in addition to $CD4^+$ T cells, taurine has been shown to modulate the function of macrophages and dendritic cells. It was reported that taurine facilitated IFN regulatory factor 7 phosphorylation and enhanced type I IFN production by reducing the ROS levels in pDCs in a neutrophil cytosolic factor 1-dependent manner [12].Huang et al. found taurine inhibited the activation of the PI3K-Akt-mTOR signaling pathway to promote the modulate of macrophage polarization [39] and regulate macrophages inflammatory pathways [40]. Our result showed taurine could reverse TLR7-mediated lupus pathogenesis, which demonstrated that taurine exerted a protective effect against SLE, suggesting that taurine supplementation may provide clinical benefits to lupus patients, the optimal concentration of taurine and the specific molecular mechanisms underlying its protective effective would be explored in the future work.

There are also some limitations in this study. Almost all of the SLE patients were on glucocorticoid, which may have an interaction with taurine, despite the fact that we did not find differences in taurine levels in different group of patients with varied glucocorticoid dose. Besides, we didn't dynamic monitor the taurine contents, while the measurement of dynamic changes of taurine in SLE patients and analysis of their correlation with disease activity and severity, as well as the drugs patients used, could provide important clue on the critical roles of taurine in the evolution of lupus. Consequently, the use of preclinical cohorts and longitudinal lupus cohorts, which include patients at different stages of the disease, will facilitate further elucidation of this issue. As the taurine transporter gene [41], $CD4^+$ T cells-specific $Taut^{-/-}$ mice also help to elucidate the exact role of taurine in SLE.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12986-025-00936-x.

Supplementary Material 1

Supplementary Material 2

Author contributions

X.F., and S.L. designed the study. S.H. and Y.C. collected the data, performed the experiments and performed the statistical analysis. Y.Z. and H.D. analyzed the data. S.H. and X.F. wrote the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

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