Berberine Modulates Lupus Syndrome via the Regulation of Gut Microbiota in MRL/Lpr Mice

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Research article

Abstract

Background

Intestinal flora disorder and immune abnormalities have been reported in systemic lupus erythematosus (SLE) patients. Few researches indicated the intestinal status in Chinese SLE patients. Berberine (BBR) showed significant effects on regulating the intestinal flora, repairing gut barriers and regulating immune cells. This study mainly explored intestinal flora and metabolites in local Chinese SLE patients and the influence of BBR to MRL/Lpr mice.

Methods

16S high-throughput sequence and gas chromatographic technique were used to analyzed the intestinal flora and metabolites in SLE patients. MRL/Lpr mice were oral treated with BBR in low, medium and high dosages for 6 weeks. Urine protein was monitored. After the procedure, gintestinal status of MRL/Lpr mice were analyzed like human. A wide range of autoantibodies were tested. Kidney tissue was analyzed for C3, IgG and IgM expressions and colon tissue was analyzed for gut barrier markers. Flow cytometry determined the immune cells.

Results

Dysbacteriosis and abnormal metabolism influenced the Chinese lupus pathogenesis. BBR treatment reduced the urine protein, inhibited the auto-antibodies and ameliorated lupus nephritis (LN) in MRL/Lpr mice. In addition, BBR altered the relative abundance of *Bacteroides* and *Verrucomicrobia* and the butyric acid content in colon of MRL/Lpr mice. The increase of tight junction protein also indicated that the gut barrier was repaired by BBR. Treg and Tfr cells in spleen and mesenteric lymph node (MLN) were increased.

Conclusions

These results revealed a therapeutic effect of berberine on SLE from gut microbiota to immune status.

1. Introduction

Systemic Lupus Erythematosus (SLE) is a prototypic autoimmune disease defined by autoantibody formation, histiocytic infiltration and terminal organ damage. Lupus nephritis (LN) is the most severe organ manifestations and the leading cause of lupus mortality [1, 2]. The hyperactivity of autoimmune T and B cells leads to immunoreactions with autoantibodies generation and systemic inflammatory response. Glomerular deposition of autoimmune complexes is the most pathological change in SLE. Systemic inflammatory activation results in acute or chronic systemic inflammatory symptoms, such as

gastrointestinal reactions. Thus, down-regulation of activated T and B cells is a legitimate way for SLE treatment [3–5].

The thriving development of microbiota research have shown the significantly abnormal microflora structure in SLE patients, such as the increased proportion of *Bacteroides* and lower *Firmicutes/Bacteroidetes* (F/B) ratio [6–8]. In the study including 61 SLE patients, the microflora was significantly correlated with SLE disease activity index (SLEDAI) [8]. High levels of short-chain-fatty acids (SCFAs) from fecal samples of 21 patients were observed, especially the acetate and propionate [9]. *Lactobacillus* treatment targeting intestinal flora of MRL/ Lpr mice significantly alleviated SLE disease manifestations. The "leaky" gut situation was eased by the increased expression of barrier-tight-function protein [10]. These researches suggested the potential role of gut microbiota in lupus pathogenesis. Meanwhile, modulating gut microbiomes can be a potential therapeutic method to SLE.

Berberine (BBR) is an important natural isoquinoline alkaloid extracted from coptis chinensis and cypress needles [11]. BBR exhibits its surprising therapeutic effects in the regulation of immunoregulation. Meanwhile, intestinal flora plays an essential role in the metabolism of BBR. In the treatment of obese rats [12], BBR showed the ability of significantly enriching the SCFA-producing bacteria which is represented by *Bacteroides*. When used to relieve the symptoms of colitis [13, 14], the metabolite of BBR increased the abundance of *Bacteroides* and regulated intestinal epithelial barrier dysfunction by enhancing the tight junction proteins, ZO-1 and occludin. BBR also increased the proportion of Foxp3^{+} Treg cells in the spleen and mesenteric lymph nodes (MLN). These results demonstrated that BBR ameliorated diseases via modulating intestinal flora and its metabolites, protecting colonic integrity, and regulating immune cells. Given the existing studies, BBR may be a promising agent worthy to be explored.

In the current study, for the first time, we analyzed the intestinal flora and metabolites of Chinese SLE patients. Meanwhile, we creatively apply BBR to SLE treatment, monitoring the effect of ameliorating intestinal dysbacteriosis to multiple organ damages and over-activated immune system. Our findings provide new insights into the treatment of autoimmune diseases.

2. Materials And Method

2.1 Research participants and sample collection

This study included stool samples from 104 SLE patients and 90 health controls from Affiliated Hospital of Nantong University during June 2017 to February 2019. Informed consent was obtained from each participant. All patients met the 2009 American College of Rheumatology (ACR) classification criteria for SLE [15]. Exclusion criteria were listed as follows: 1) Pregnancy or breast-feeding; 2) Recent or current medical disorder (cardiac, respiratory, gastrointestinal, neurological, endocrine, malignancy, etc); 3) History of probiotics within 2 weeks or antibiotics within 3 months before admission; 4) If on immunosuppressant, the dose must be stable for 4 weeks before the sample collection. The gender and age-matched healthy controls (N) were recruited from the Health Examination Centre of Affiliated Hospital

of Nantong University. The inclusion of human participants and supporting documentation were approved by the Affiliated Hospital of Nantong University Ethics Committee (2017-K002). All fecal specimens of participants were collected in a sealed fecal storage box after defecation and stored at -80°C after collection.

2.2 Experimental animal and treatments

Fourty six-week-old female MRL/Lpr mice and ICR mice were bought from Shanghai Sushang Biological Technology Co., LTD. The mice were in the SPF environment of animal research center of Nantong University and were housed three to four per cage at 23–24 °C with a 12-h/12-h light/dark cycle with free access to food and water. This animal study was proved by the Institutional Animal Care and Use Committee of Nantong University (S20200313-014).

These mice were divided into five groups (8 mice/group): control ICR mice (N) and MRL/Lpr mice (S) were treated with saline. The dose of BBR in person is 0.3g-0.9g/day. According to Meeh-Rubner equation [16], MRL/Lpr mice (L, M and H) were treated with BBR in the daily amount of 60mg/kg, 120mg/kg and 180mg/kg. Urine was collected for 24 h and detected by the Bradford Protein Assay Kit (Solarbio, Beijing). After the appearance of lupus nephritis (Fig. 3B), mice were given saline or berberine orally. After 6 weeks' treatment, mice were sacrificed. Serum, faeces and organs samples were collected. The kidneys and colons were formalin-fixed and paraffin-embedded and sliced at 4 μm thickness for further staining. Serum antibody level (dsDNA, ANA, C3, C4) in mice was detected by ELISA (Jingmei Biotechnology, China). The entire progress was showed in Fig. 3A.

2.3 16S high-throughput (16S rRNA) sequencing and short-chain fatty acid detection

16S rRNA sequencing of intestinal flora in intestinal faeces was completed in Sangon Biotech (Shanghai) Co., LTD. Microbial DNA was extracted by QIAamp® Fast DNA Stool Mini Kit (QIAGEN, Germany). The V3-V4 hypervariable regions of the microbiota 16S rRNA gene were amplified with primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3').

Data analyses were performed by the Sangon platform. The detection of intestinal metabolites, short-chain fatty acids, was carried out by Wuhan Huada Medical Laboratory Co. LTD (supplementary Fig. 1). Thermo Trace1300-Thermo TSQ9000 tandem mass spectrometry and SIM mode were used for forward detection. Tracefinder (Thermo Fisher Scientific, Waltham, MA, USA) was used for data processing. After calculation, the absolute content of target compounds in samples was obtained. The datasets used and analysed during the study are available from the corresponding author.

2.4 Extraction of total DNA of fecal sample and quantitative real-time Polymerase Chain Reaction (qRT-PCR).

The QIAamp® Fast DNA Stool Mini Kit (QIAGEN, Germany) was used to obtain the total DNA from the sample of faeces. The DNA sample was diluted with RNase free water (Beyotime, China) into 5ng/ul and performed the real-time quantitative PCR. According to the bacterial colony 16S rRNA V3 sequence, the specific primers of total bacteria, *Bacteroides* and *Firmicutes* were designed and synthesized by Biomics Biotechnology, China. Sequences and reaction systems were showed in supplementary Tables 1 and 2. The procedure was operated in the Roche cobas z 480.

2.5 Histo-morphological Staining

The colon and kidney sections were stained with hematoxylin-eosin (HE), periodic acid-Schiff (PAS) and Masson's trichrome respectively (Solarbio, Beijing). HE staining monitored the histological change, PAS staining evaluated the inflammatory cell infiltration and Masson's trichrome staining determined the degree of fibrosis. Pathological histology was observed by a biological microscope.

2.6 Immunofluorescence

Kidney slides were dewaxed and incubated with antigen retrieval solution and 3% H_2O_2 (Solarbio, China). The kidney tissues were blocked by gout serum (Maxim,China). For direct immunofluorescence, sections were labeled with anti-C3 antibody (ab11862, Abcam), anti-IgM antibody (ab150121, Abcam) and anti-IgG antibody (ab150083, Abcam) for 30–60 min at room temperature. DAPI was used to stain the cell nuclei. Immunofluorescence staining of colon sections were similar to the above steps, with antibodies like anti-ZO-1 antibody (ab216880, Abcam) and anti-occludin antibody (ab216327, Abcam).

2.7 Western blot

Total protein was extracted from colon tissues using protein lysate (RIPA: PMSF = 100:1, Beyotime, China) and determined by a BCA Protein Assay kit (Beyotime, China). Proteins blotted with antibodies to ZO-1 (ab216880, Abcam, China) and occludin (ab216327, Abcam, China). ß-actin (110007, CST, China) served as the internal control. An enhanced chemiluminescence kit (ECL, Millipore, USA) was used to display the blots.

2.8 Flow cytometry

Lymphocytes were isolated from the tissue homogenates of spleen and mesenteric lymph nodes (MLN). The following primary antibodies were used in the process: mouse BV510-CD3 (Cat. 100233, BioLegend), FITC-CD4 (Cat. 100405, BioLegend), APC-CD25 (Cat. 101909, BioLegend), PE-Cy7-CXCR5 (25-7185-82, eBioscience), PE-Foxp3 (12-5773-82, eBioscience), FITC-CD21 (Cat. 123407, BioLegend), PE-Cy7-CD23 (25-0232-82, eBioscience), Alexa Fluor 700-CD19 (Cat. 115527, BioLegend), BV785-CD25 (Cat. 102051, BioLegend), BV510-CD3 (Cat. 100353, BioLegend), APC-NK1.1 (17-5941-82, eBioscience). Cell subsets were analysed on a BD LSRFortossaTM flow cytometer (BD Biosciences, USA) with FACSDiva Software (BD Biosciences) and FlowJo Software (Tree Star Inc., USA).

2.9 Statistical Analysis

Quantitative data were expressed as mean ± S.E.M. Differences determined by two-tailed t-test were used for two-group comparisons and One-way ANOVA was used for multiple comparisons. P-values < 0.05 indicated the experimental results are reliable.

3. Results

3.1 The intestinal flora and metabolites influenced the disease activity of Chinese SLE patients.

In our study, 16S high-throughput sequencing were conducted in faeces from 40 SLE patients and 26 normal people. There was no significant difference between community richness (Fig. 1A) and diversity (Fig. 1B) in two groups. Gut microbiota in two groups showed the similar composition (Fig. 1C). It is noteworthy that SLE patients showed higher abundance of *Bacteroidetes* and lower abundance of *Firmcutes* (Fig. 1C). Similar flora composition showed in the MRL/Lpr mice which indicated the MRL/Lpr mice could be used as dysbacteriosis model of SLE (Fig. 5C).

Then, Stool samples from 64 normal people and 64 SLE patients were collected to validated the above conclusion. The results of qRT-PCR showed higher *Firmcutes*/*Bacteroidetes* (F/B) ratio in SLE patients (Fig. 1D). The F/B ratio was negatively correlated with SLEDAI score (Fig. 1E). These results substantiated the dysbacteriosis in SLE patients and its relationship in disease development.

Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) technique was applied to identify functional categories (Fig. 2). And the related functions such as immune system diseases, membrane transport, amino acid metabolism were quite different between two groups. Gas chromatography technology was applied to analysis the metabolism (Table 1). Almost all the tested SCFAs found statistically significant results in SLE and LN patients, especially the propanoic acid and butyric acid. We could conclude that dysbacteriosis and abnormal metabolism can influence Chinese lupus pathogenesis.

Table 1

Analysis of fecal short-chain fatty acids in healthy controls and SLE patients with different disease activity. The differences in specific short-chain fatty acids between healthy controls (N), SLE patients (S) and SLE patients with lupus nephritis (LN) were assessed by gas chromatography-mass spectrometry (GC-MS). N: n = 26, male: female = 1: 25, 38.25 ± 14.32 years. S: n = 23, male: famale = 0:23, 40.30 ± 2.796 years. LN: n = 17, male: famale = 1: 16, 31.71 ± 2.405 years. a Unpaired t test. P values in bold are < 0.05. Variables are summarized as mean ± S.E.M.

Short-chain-fatty acids(ng/mg)	N (n = 26)	S (n = 23)	LN (n = 17)	P value[a]		
				N vs S	N vs LN	S vs LN
Acetic acid	4.0 ± 1.04	4.2 ± 0.98	3.6 ± 1.00	0.8993	0.8035	0.6999
Propanoic acid	105.7 ± 30.89	40.2 ± 10.52	154.2 ± 44.50	**0.0471**	0.3618	**0.0070**
Butyric acid	207.2 ± 53.25	68.5 ± 21.22	267.1 ± 59.71	**0.0243**	0.4643	**0.0014**
Isobutyric acid	18.6 ± 2.57	26.5 ± 6.32	38.4 ± 7.00	0.2459	**0.0049**	0.2204
N-Valeric acid	92.3 ± 17.38	75.2 ± 19.07	193.0 ± 54.78	0.5126	**0.0469**	**0.0336**
Isovaleric acid	42.2 ± 5.71	47.1 ± 9.87	93.3 ± 19.62	0.6578	**0.0066**	**0.0325**
Caproic acid	23.3 ± 6.68	29.6 ± 6.13	24.7 ± 7.32	0.4868	0.8859	0.6064

3.2 Berberine treatment alleviated the disease symptoms of MRL/Lpr mice.

In this study, berberine was used to treat the animal model of SLE- MRL/Lpr mice. At the end of procedure, body weight was not statistically different between BBR treatment and saline treatment (Fig. 3C). The decrease of urine protein suggesting the relieving effect of berberine on lupus nephritis (Fig. 3D). The concentration of dsDNA and ANA antibodies was increased in the serum of BBR treated MRL/Lpr mice, while the complement C3 and C4 was decreased (Fig. 3E-H). Next, we assessed renal histopathological changes (Fig. 4A). The HE, PAS and Masson staining results demonstrated serious mesentery proliferation, inflammatory cell infiltration and renal interstitial in MRL/Lpr mice. We found that BBR gradually alleviated kidney pathological changes in MRL/lpr mice in a dose-depended manner. The immune complex-induced vascular inflammation are key mechanisms in the development of LN [17]. The classical generation of C3 is originated by C1q directly binding to complement-fixing antibodies- IgM and IgG. Glomerular deposition of C3, IgG and IgM were significantly high in MRL/Lpr mice (Fig. 4B). The deposition of complement C3 and immunoglobulin IgM and IgG in all BBR treatment groups were significantly reduced compared with control MRL/Lpr mice. These results indicated that BBR treatment

relieved autoantibody secretion, alleviated nephritis conditions and ameliorated the renal pathological damage of MRL/Lpr mice.

3.3 Berberine regulated intestinal flora and metabolites in MRL/Lpr mice.

It has been reported that berberine regulated intestinal flora as an anti-inflammatory agent, especially increasing the proportion of *Bacteroides* [18, 19]. MRL/Lpr mice showed increasing gut microbiota richness and diversity, while BBR significantly abrogated the differences in MRL/lpr mice (Fig. 5A, B). At the phylum level (Fig. 5C), *Bacteroidetes* and *Verrucomicrobia* was increased gradually in MRL/lpr mice treated with BBR at all dose. *Firmicutes* was decreased distinctly in the MRL/lpr mice treated with BBR at medium and high dose, where *Bacteroidetes* became the dominant bacteria. As the result of flora adjustment, the expression of major SCFAs in fecal sample surprisingly increased, especially the butyric acid and n-valeric acid (Table 2).

Table 2
Berberine enhance the expression of butyic acid and N-Valeric acid in MRL/Lpr mice.

The differences in specific short-chain fatty acids between five groups of mice were assessed by gas chromatography-mass spectrometry (GC-MS). a one-way ANOVA. P values in bold are < 0.05. Variables are summarized as mean ± S.E.M.

Short-chain-fatty acids (ng/mg)	N (n = 8)	S (n = 8)	L (n = 8)	M (n = 8)	H (n = 8)	P value[a]			
						S vs N	S vs L	S vs M	S vs H
Acetic acid	8.3 ± 1.58	4.4 ± 0.98	5.5 ± 0.78	8.6 ± 3.10	5.8 ± 0.80	0.0558	0.3843	0.2121	0.2948
Propanoic acid	7.63 ± 0.82	6.8 ± 0.62	7.1 ± 0.64	9.6 ± 1.84	7.4 ± 0.65	0.4098	0.6800	0.1611	0.4988
Butyric acid	19.8 ± 2.19	11.4 ± 1.89	19.0 ± 4.21	36.5 ± 15.71	20.4 ± 3.47	**0.0117**	0.1206	0.1345	**0.0389**
Isobutyric acid	6.6 ± 0.80	6.5 ± 0.73	7.9 ± 1.63	8.0 ± 1.36	6.8 ± 0.648	0.9099	0.4545	0.3487	0.8018
N-Valeric acid	37.4 ± 7.45	14.5 ± 1.95	39.4 ± 8.82	46.0 ± 19.96	41.1 ± 8.10	**0.0101**	0.0155	0.1385	**0.0065**
Isovaleric acid	16.4 ± 4.17	9.8 ± 1.09	18.1 ± 3.44	21.4 ± 6.25	13.4 ± 2.82	0.1464	0.0883	0.2502	0.2502
Caproic acid	4.9 ± 0.23	4.3 ± 0.17	4.4 ± 0.18	5.1 ± 0.48	4.1 ± 0.13	0.0694	0.6525	0.1226	0.3430

3.4 Berberine enhanced intestinal barrier function in MRL/Lpr mice.

Mechanical barrier refers to the intact intestinal mucosal epithelial cells and the tight connections between the epithelial cells [20, 21]. The main manifestations of MRL/Lpr mice were destruction of epithelial structure and goblet cells. The intervention of berberine completed the intestinal tissue structure tends, rearranged goblet cells and reduced the intestinal inflammation, especially in MRL/Lpr mice treated with high BBR dose (Fig. 6A).

Western blot was used to analysis the protein ZO-1 and occludin (Fig. 6B, C). The MRL/Lpr mice showed significant deficiency of both proteins, while the protein expression level of ZO-1 increased significantly in group M and H and the expression of occludin increased in all BBR-treated mice. Immunofluorescence staining showed that MRL/Lpr mice were also deficient in related proteins, while in all BBR-treated groups, ZO-1 and occludin were significantly enhanced (Fig, 6D). The application of berberine enhance the intestinal barrier function.

3.5 Berberine regulated immune function in MRL/Lpr mice.

Immune manifestation of SLE is the most direct embodiment of the abnormal activation of the immune system. The abnormal activation of T and B cells directly leads to the harmful changes in immune functions [22–25]. As shown in Fig. 7A, B, the decreased spleen index and lymph nodes index directly showed the amelioration of immune activation in the BBR-treated group. Smaller spleen size was quite obviously in group M and H (Fig. 7C).

Lymphocytes from MLN and spleen were obtained and detected by flow cytometry. Statistical results indicated that Treg and Tfr cells were increased in the MRL/Lpr spleen after the berberine treatment (Fig. 7D, F). Meanwhile, the proportion of Treg cells in MLN was enhanced (Fig. 7E, G). However, no significant results were found for B cells and NK cells (supplementary Fig. 2, 3).

4. Discussion

Previous studies have reported interactions between the gut microbiota and alterant homeostatic balance in SLE patients. Further researches revealed the correlation between the abnormal abundance of the *Firmcutes* and *Bacteroidetes* and disease activity in SLE [8, 26]. Gut microbiota are associated with the pathogenesis of disease through intestinal metabolites- SCFAs [27, 28], While few reports mentioned the abnormal gut metabolites in SLE patients [9]. Our investigation, for the first time, illustrated the relationships between gut microbiota, intestinal metabolites and disease activity in Chinese SLE patients.

In MRL/Lpr mice, berberine significantly eased splenomegaly and increased Treg, Tfr in spleen and MLN. Abnormal autoantibodies in the circulation and kidney damage changed, too. Metabolism of berberine is confined in the gut lumen [29]. Increasing evidences showed that intestinal microbiota is the target of berberine [30]. Like in colitis mice, BBR increased the abundance of *Bacteroidetes* and ameliorated

intestinal epithelial barrier function via enhancing the expressions of tight junction proteins (ZO-1 and occludin) in MRL/Lpr mice. As a member of butyrate-producing communities [31], increasing of *Bacteroidetes* leads to the production of butyric acids. Butyrate showed the ability of enhancing the intestinal barrier [14, 32, 33] and facilitating the generation of Treg cells [34, 35]. Therefore, the repaired "leaky" gut and a shift of immune balance could explain how berberine remitted lupus disease in MRL/Lpr mice.

The gastrointestinal tract is the largest immune organ which leading the regulation of immune homeostasis. Intestinal epithelial barrier showed strong interactions with the gut microbiome and immune system [36]. Recent studies indicated composition of the gut microbiome modulated metabolites, which affect the gut barrier. It is noteworthy that intestinal barrier disruption can be related to increased susceptibility to immune diseases [20, 37]. Current studies confirmed the dysregulation of intestinal flora and metabolites (SCFAs) in SLE. Decreased tight junction protein [38] and increased circulating endotoxin [26] indicated the change of intestinal permeability in MRL/Lpr mice and SLE patients. Oral BBR treatment in MRL/Lpr mice altered the proportion of *Bacteroidetes* and butyric acids, which directly repaired the intestinal epithelial barrier.

Treg cells prevent autoimmune diseases and maintain a stable immune state [39].Treg cells were considered to be inhibitors of lupus disease, which resulted in delayed disease progression and reduced mortality in lupus prone mice [40]. Increasing Treg cells proportion was observed with Berberine treatment [41, 42]. Butyrate promotes differentiation of Foxp3$^+$ Treg cells in *vivo* [34]. Tfr cells compete intensively with Tfh on germinal center B cells, promoting the production of high-affinity antibodies and limiting the overall expansion of antigen-specific B cell clones [43]. In mice model, the loss of Tfr cells leads to the proliferation of Tfh and GCB cells, as well as the production of antibodies [44, 45]. Those fact may explain the increased Tfr cells in berberine treated mice.

Together with the change of immune cells, BBR treatment do improve lupus nephropathy in MPL/lpr mice. The level of anti-dsDNA antibodies can reflect disease activity, while antinuclear antibodies (ANAs) mediated tissue deposition of immune complexes [2, 46]. Serum C3 and C4 were correlated with disease activity to some degree [47]. Low C3 was associated with renal involvement and poor renal prognosis [48]. Our results showed that BBR was able to ease nephropathy in MRL/Lpr mice as reducing level of urine protein, serum immunological markers and improving kidney function. In addition, BBR was able to down-regulate the urine protein, which indicated increase proximal tubular reabsorption and glomerular filtration rate. Furthermore, BBR show the ability of inhibiting complement cascade via preventing the consumption of serum C3 and C4. The effect of BBR on lupus nephritis was confirm by histopathological study and immunofluorescence. BBR was able to improve the damaged kidney to the relatively normal glomerular structure and inhibit the levels of local C3, IgG and IgM.

Our study highlights current status of intestinal dysbacteriosis in Chinese patients with SLE and differences in intestinal metabolites among patients with different disease states. The regulation of

intestinal flora and the repairment of gut barrier by intestinal metabolites in BBR treated mice seemed to be the factor that directed the immune responses and disease outcomes (Fig. 8).

5. Conclusions

In conclusion, gut microbiome regulated the disease development in Chinese SLE patients. Also, BBR treatment modulates the lupus syndrome via the regulation of gut microbiota. The application of berberine is a relatively safe and convenient way. However, it is difficult to promote this method due to the complexity of individual flora and diverse microflora in different disease activities. Therefore, further investigations will focus on the effects of berberine and its metabolites on intestinal function and systemic immunity.

6. Abbreviations

SLE
systemic lupus erythematosus; BBR:Berberine; LN:Lupus nephritis; MLN:Mesenteric lymph node; F/B:Firmicutes/Bacteroidetes; SLEDAI:SLE disease activity index; SCFAs:Short-chain-fatty acids; Treg:Regulatory T cells; Tfh:T follicular helper cells; GCB:Germinal center B cells; dsDNA:Double-stranded DNA; ANA:Antinuclear antibody; C3:Complement 3; C4:Complement 4; HE:hematoxylin-eosin; PAS:periodic acid-Schiff; H_2O_2:Hydrogen peroxide; RIPA:RIPA Lysis buffer; PMSF:Phenylmethanesulfonyl fluoride; BCA:Bicinchonininc acid; IgG:immunoglobulin G;

7. Declarations

7.1 Ethics approval and consent to participate

Informed consent was obtained from each participant. The inclusion of human participants and supporting documentation were approved by the Affiliated Hospital of Nantong University Ethics Committee (2017-K002) and conformed to the ethical guidelines of the Declaration of Helsinki as revised in 2013. Animal study was proved by the Institutional Animal Care and Use Committee of Nantong University (S20200313-014).

7.2 Consent for publication

Not applicable.

7.3 Availability of data and materials

The data underlying this article are available in the article and supplementary material.

7.4 Competing interests

There were no competing interests between the authors.

7.6 Author' contributions

Yanfeng Bao and Yeqing Wu conceptualized the research and finished the manuscript. Yanfeng Bao and Tian Chen prepared and maintained the MRL/Lpr mice. Yanfeng Bao, Tian Chen and Yi Jin performed histomorphological staining, immunofluorescence and western blot. Xiaoqi Sha and Xingu Ge prepared the materials. Yan Meng finished experimental structure diagram. Genkai Guo, Yunfei Xia and Junling Yang discussed and supervised the experimental progress. Chi Sun and Chen Dong completed the table and figures. Juan Ji, Zhonghui Xue and Zhifeng Gu supervised the project. All authors approved the final manuscript.

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Figures
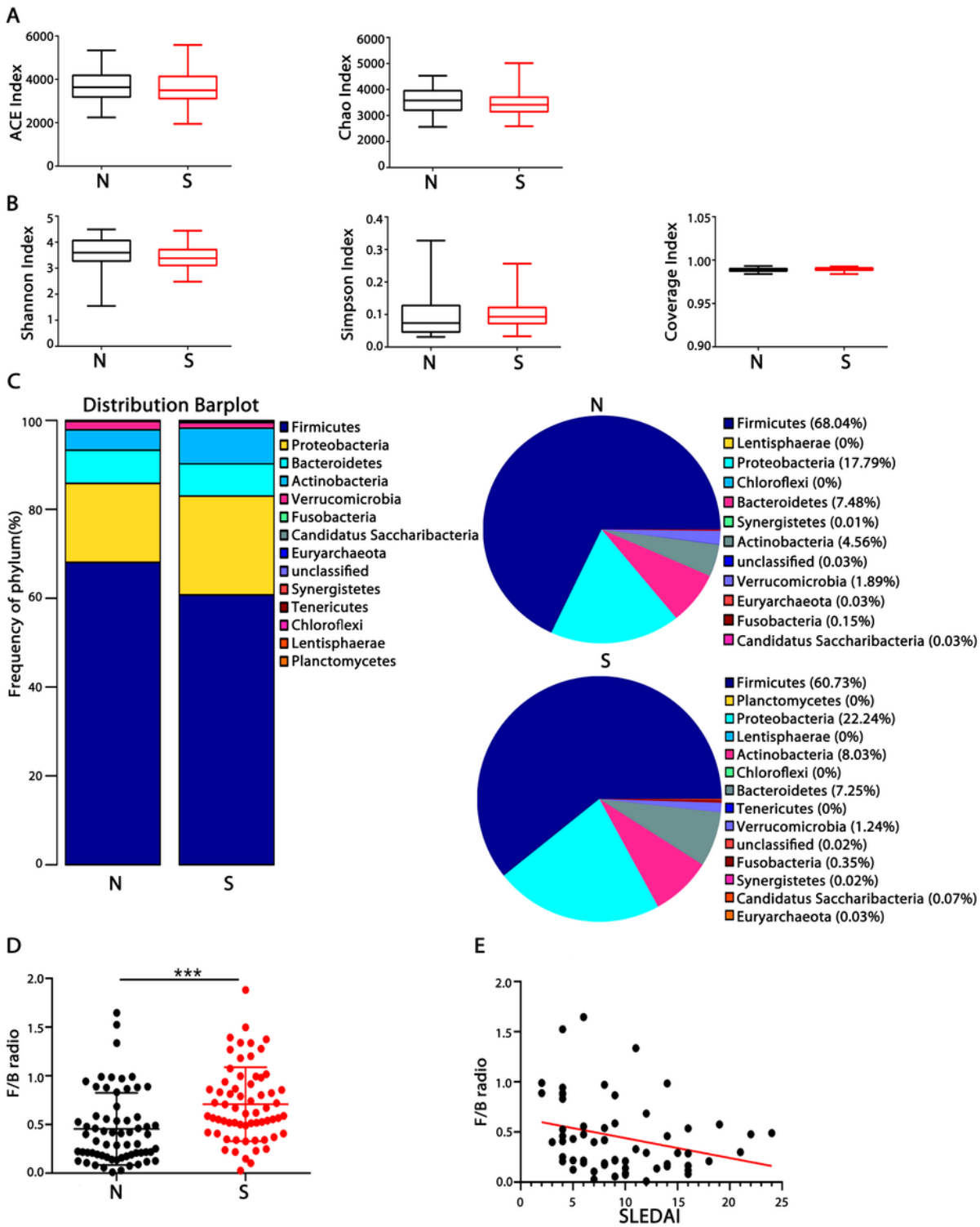
Figure 1

Gut microbiota composition differ significantly between healthy controls and SLE patients. (A) Gut microbiota richness: ACE and Chao index of faeces of healthy controls (N: n = 26, male: female= 1: 25, 38.25±14.32 years) and SLE patients (S: n = 40, male: female= 1: 39, 38.83±13.83 years). (B) Gut microbiota diversity: Shannon, Simpson and Coverage index of healthy controls (N: n = 26, male: female= 1: 25, 38.25±14.32 years) and SLE patients (S: n = 40, male: female= 1: 39, 38.83±13.83 years). (C)

Bacterial taxa at the phylum level are shown in barplot and detail percentage of bacterial taxa are shown in pie plot (healthy control, N: n = 26, male: female= 1: 25, 38.25±14.32 years. SLE patients, S: n = 40, male: female= 1: 39, 38.83±13.83 years). (D) Ratio of Firmcutes to Bacteroidetes (F/B) in faeces of healthy controls (N: n = 64, male: female= 2: 62, 38.25±14.32 years) and SLE patients (S: n = 64,male: female=2: 62,38.83±13.83 years). ***p < 0.001by two-tailed unpaired Student's t-test. (E) The association between Systemic lupus erythematosus disease activity index (SLEDAI) and F/B, R2=0.07993, P=0.0236.
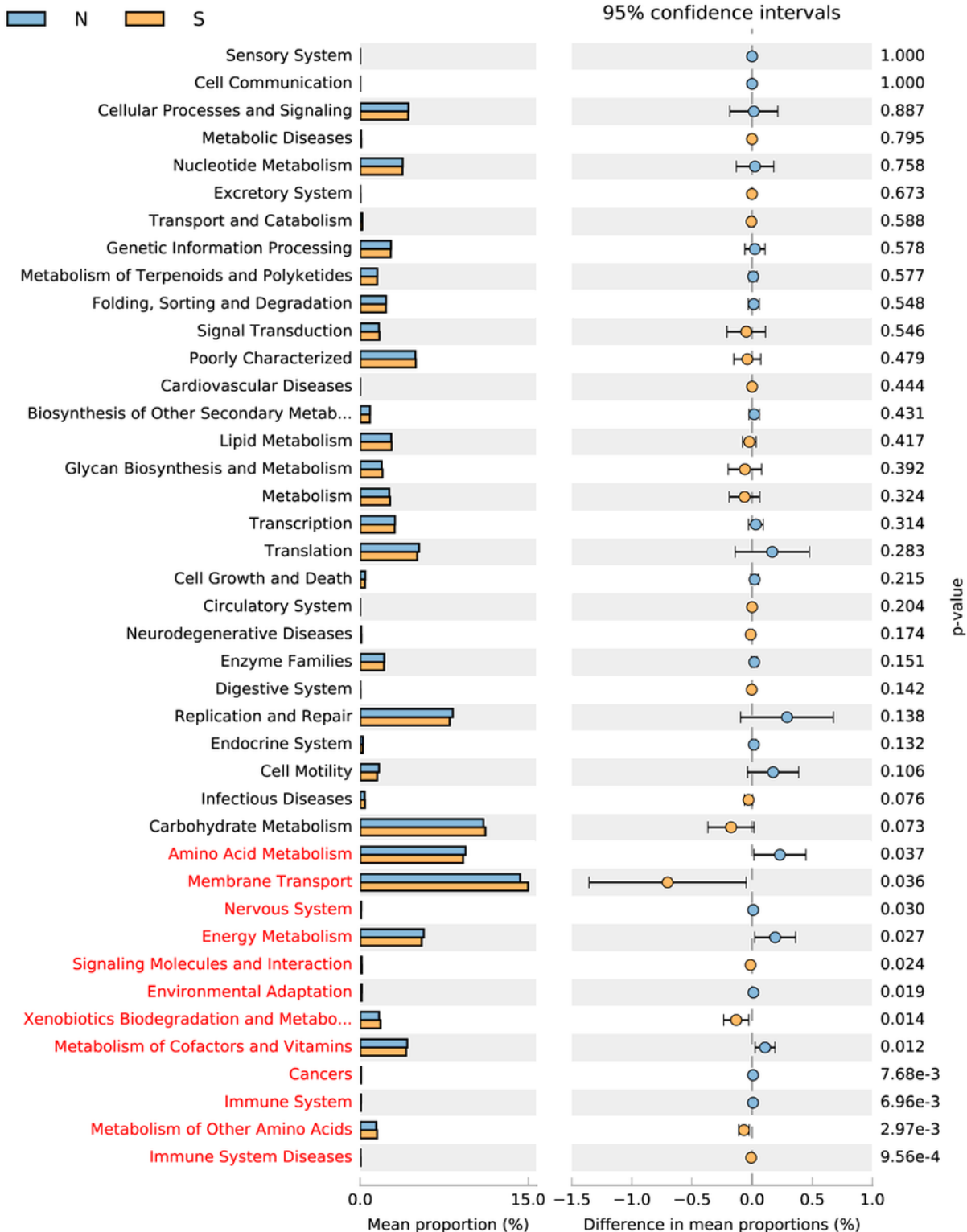
Figure 2

PICRUSt function prediction (healthy control, N: n = 26, male: female= 1: 25, 38.25±14.32 years. SLE patients, S: n = 40, male: female= 1: 39, 38.83±13.83 years).
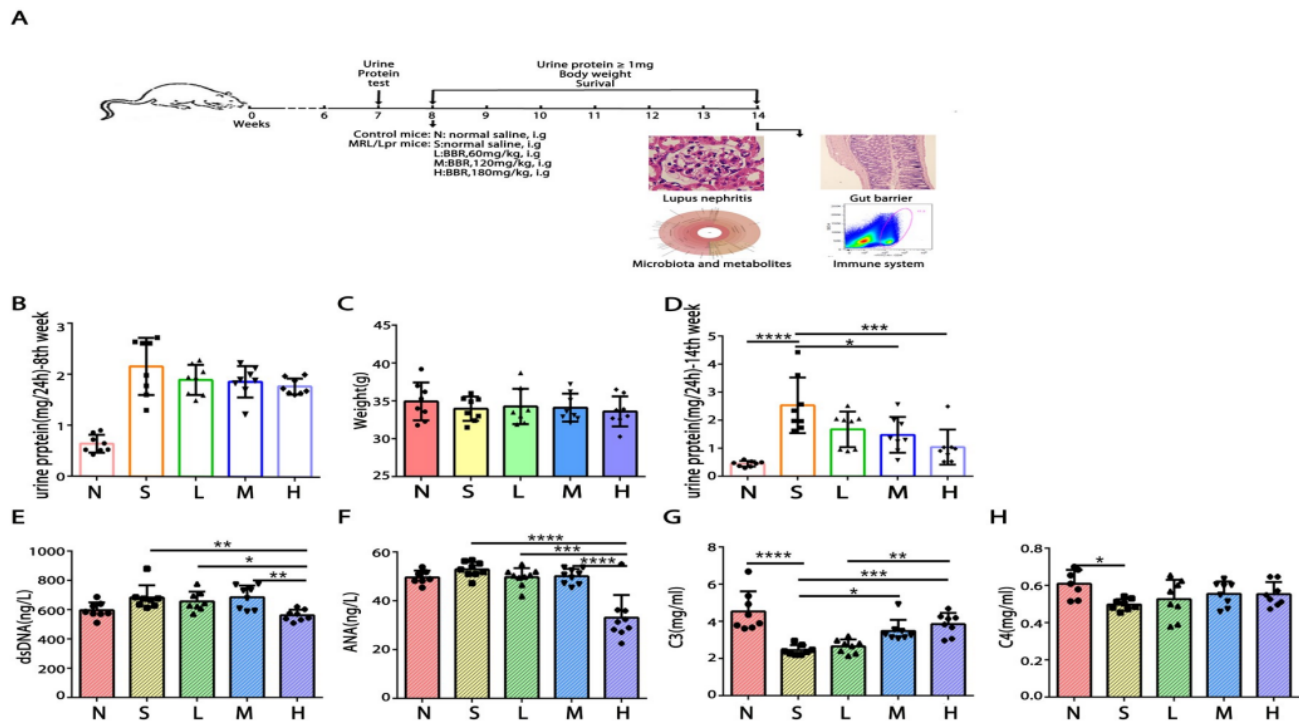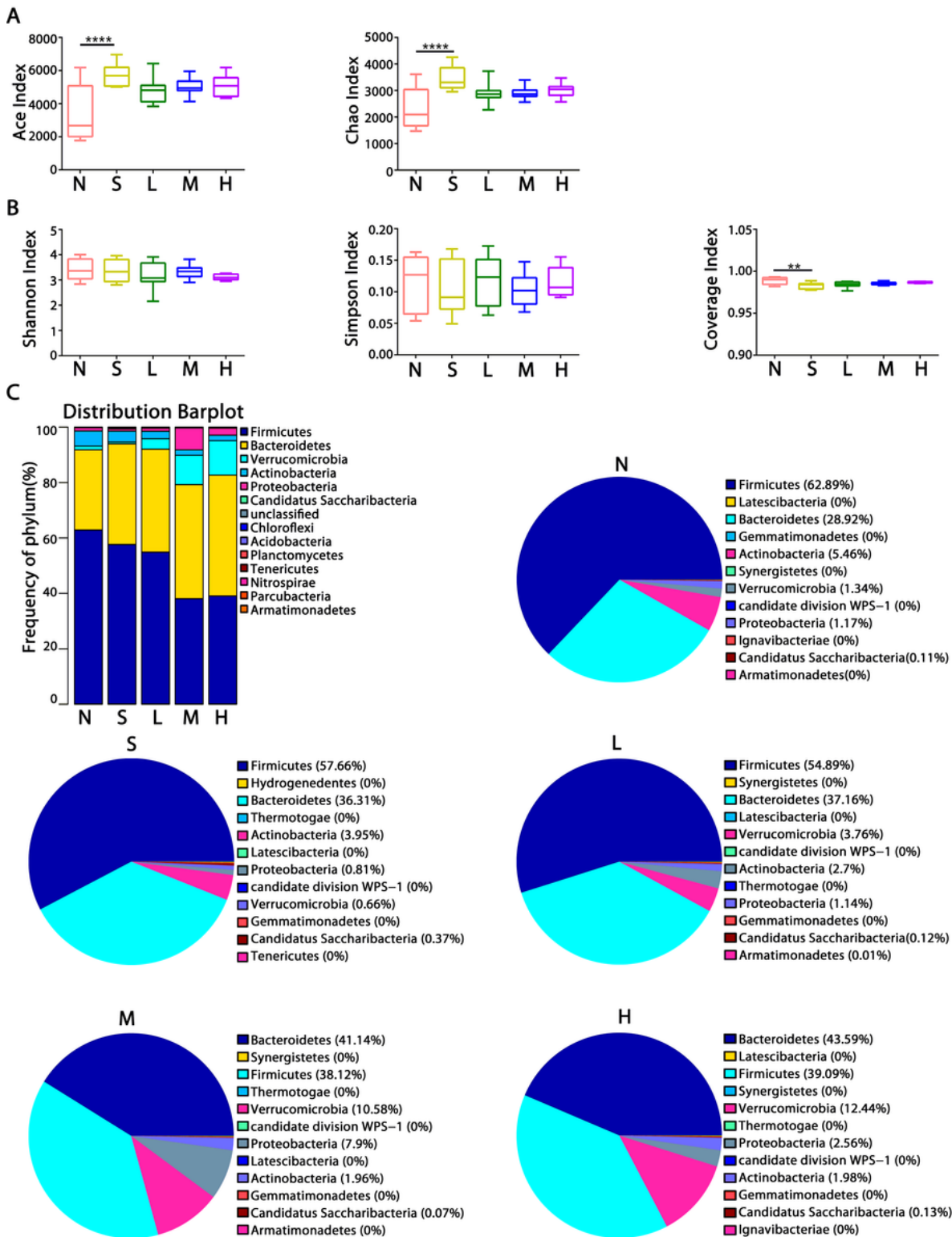

Figure 3

Berberine treatment relieves the kidney function and autoantibody secretion in MRL/Lpr mice. (A) Schematic diagram of berberine (BBR) treatment, tissue preparation and experimental protocols. The BBR treatment started at the time of the urine protein level >1mg/24h in MRL/Lpr mice. Mice were divided into five groups, 8 mice per group. Group N: control mice treated with saline, i.g; Group S: control MRL/Lpr mice treated with saline, i.g; Group L: BBR low dose MRL/Lpr mice treated with BBR 60mg/kg/d, i.g; Group M: BBR medium dose MRL/Lpr mice treated with BBR 120mg/kg/d, i.g; Group H: BBR high dose MRL/Lpr mice treated with BBR 180mg/kg/d, i.g. Mice were monitored for 6 weeks before sacrificed. Serum, faeces and tissue sample were collected for further experiment. (B) Urine protein level >1mg/24h in MRL/Lpr mice in 8-weeks-old. (C) BBR treatment do no difference to the body weight of MRL/Lpr mice. (D) BBR treatment reduced 24 h urine protein levels in MRL/Lpr mice. n = 8 for each group.*p < 0.05,**p < 0.01 by one-way ANOVA. (E-H) BBR treatment decreased the secretion of antibody dsDNA and ANA, and increased the secretion of complement C3 and C4. n = 8 for each group.*p < 0.05,**p < 0.01,***p < 0.001,****p < 0.0001 by one-way ANOVA.
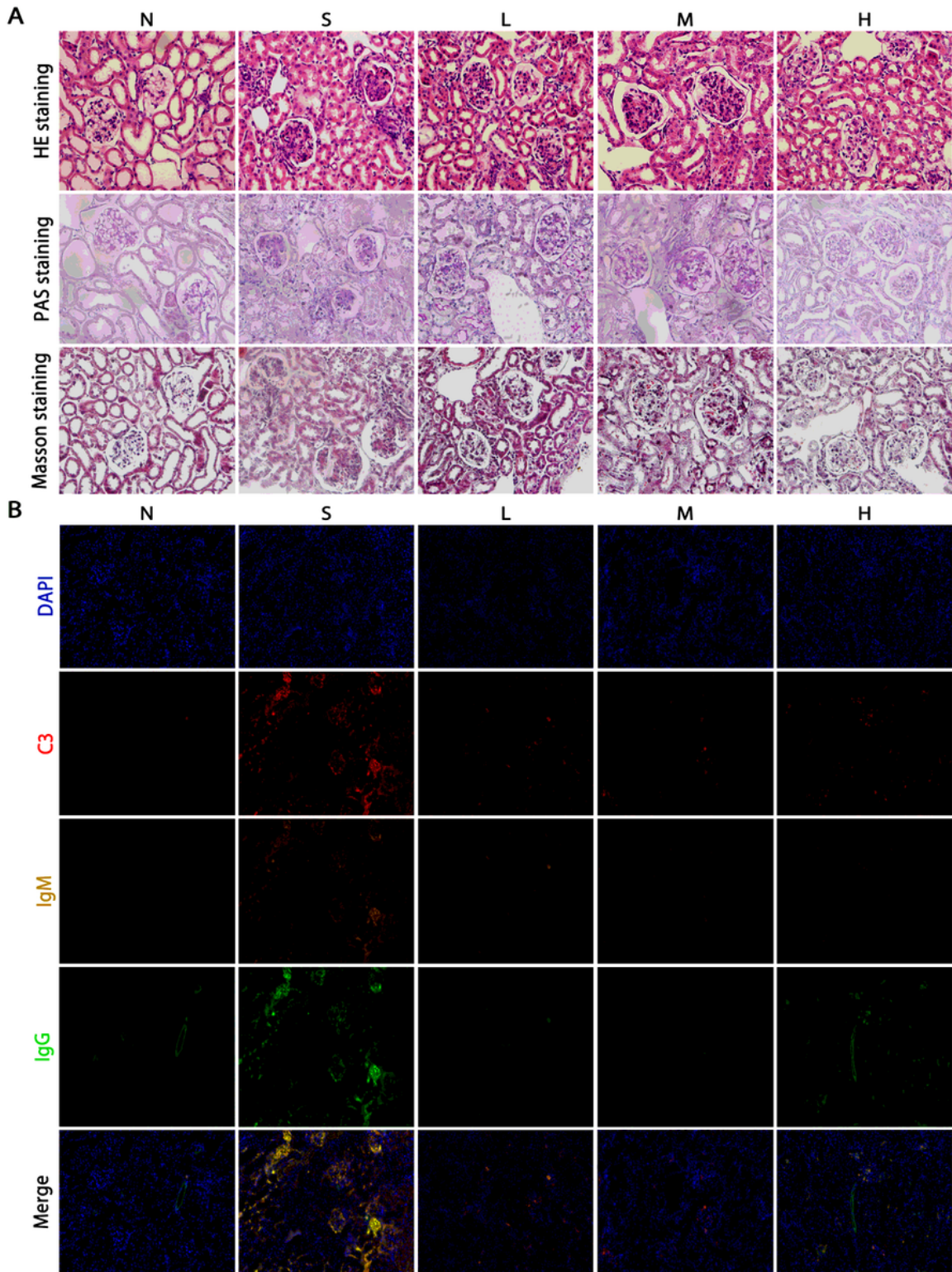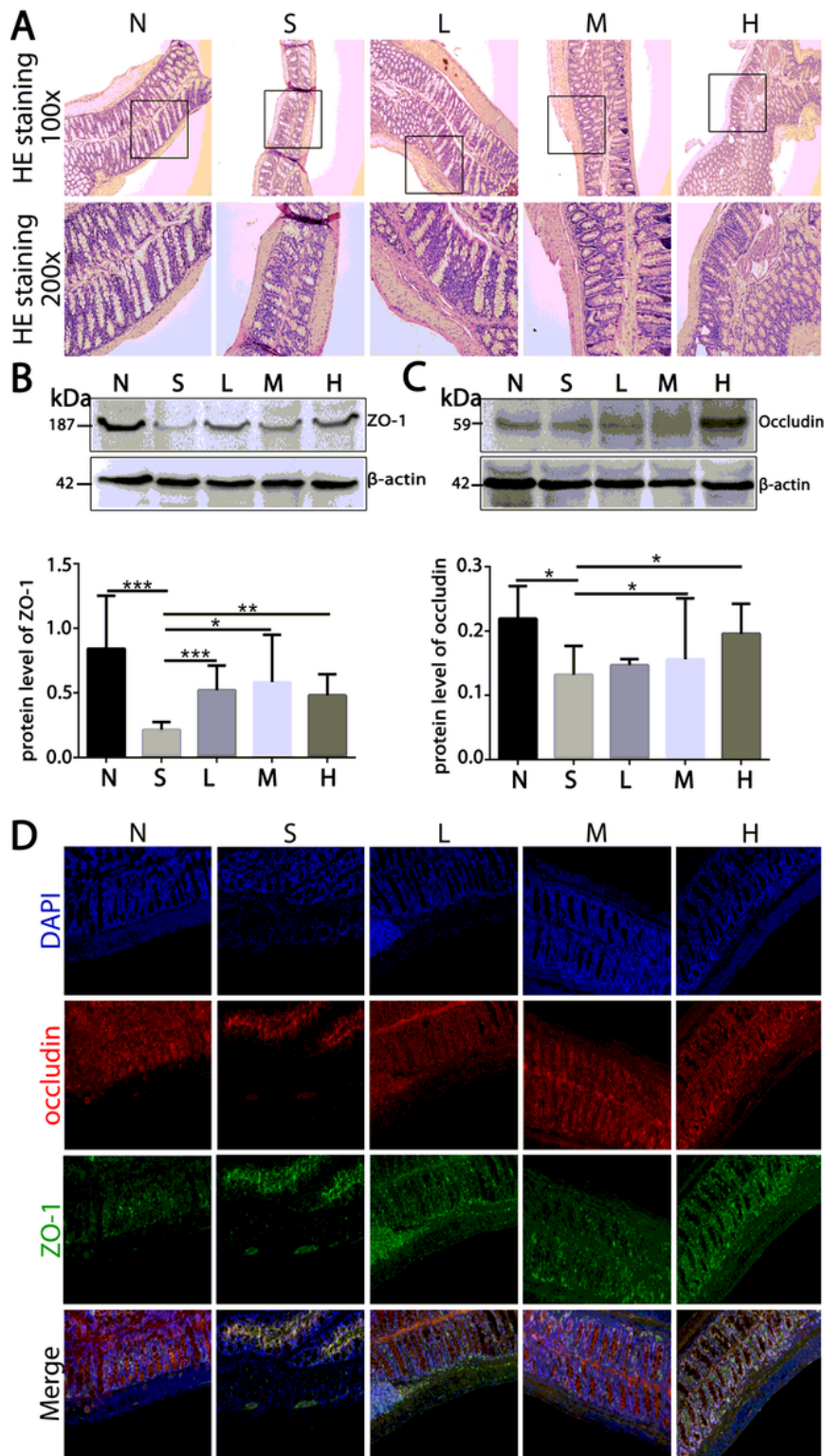
Figure 4

Berberine treatment relieves immume complex desposition in glomerulus of MRL/Lpr mice. (A) Representative images of renal sections stained with HE, PAS and Masson of five group of mice. Original magnification: ×200. (B) Immunofluorescent staining of C3 (red), IgM (yellow) and IgG (green) for renal sections from of five group of mice. Original magnification: ×200.

Figure 5

Berberine modulate gut microbiota in MRL/Lpr mice. (A) Gut microbiota richness: ACE and Chao index of faeces of five group of mice. n = 8 for each group. (B) Gut microbiota diversity: Shannon, Simpson and Coverage index of five group of mice. n = 8 for each group. (C) Bacterial taxa at the phylum level are shown in barplot and detail percentage of bacterial taxa are shown in pie plot. n = 8 for each group.

Figure 6

Responses of colonic mucosa barrier function of MRL/Lpr mice toward berberine treatments. (A) Representative images of colon sections stained with HE of five groups of mice. Original magnification: ×100 and ×200. (B) Western blot analysis of ZO-1 protein in colon tissue from five groups of mice. The same volume was loaded per lane and the levels of ZO-1 were normalized to the expression levels of β-actin. *p < 0.05, **p < 0.01, ***p < 0.001by unpaired t-test. (C)Western blot analysis of Occludin protein in

colon tissue from five groups of mice. The same volume was loaded per lane and the levels of Occludin were normalized to the expression levels of β-actin. *p < 0.05 by unpaired t-test. (D) Immunofluorescent staining of Occludin (red) and ZO-1 (green) for colon sections from of five group of mice. Original magnification: ×200.
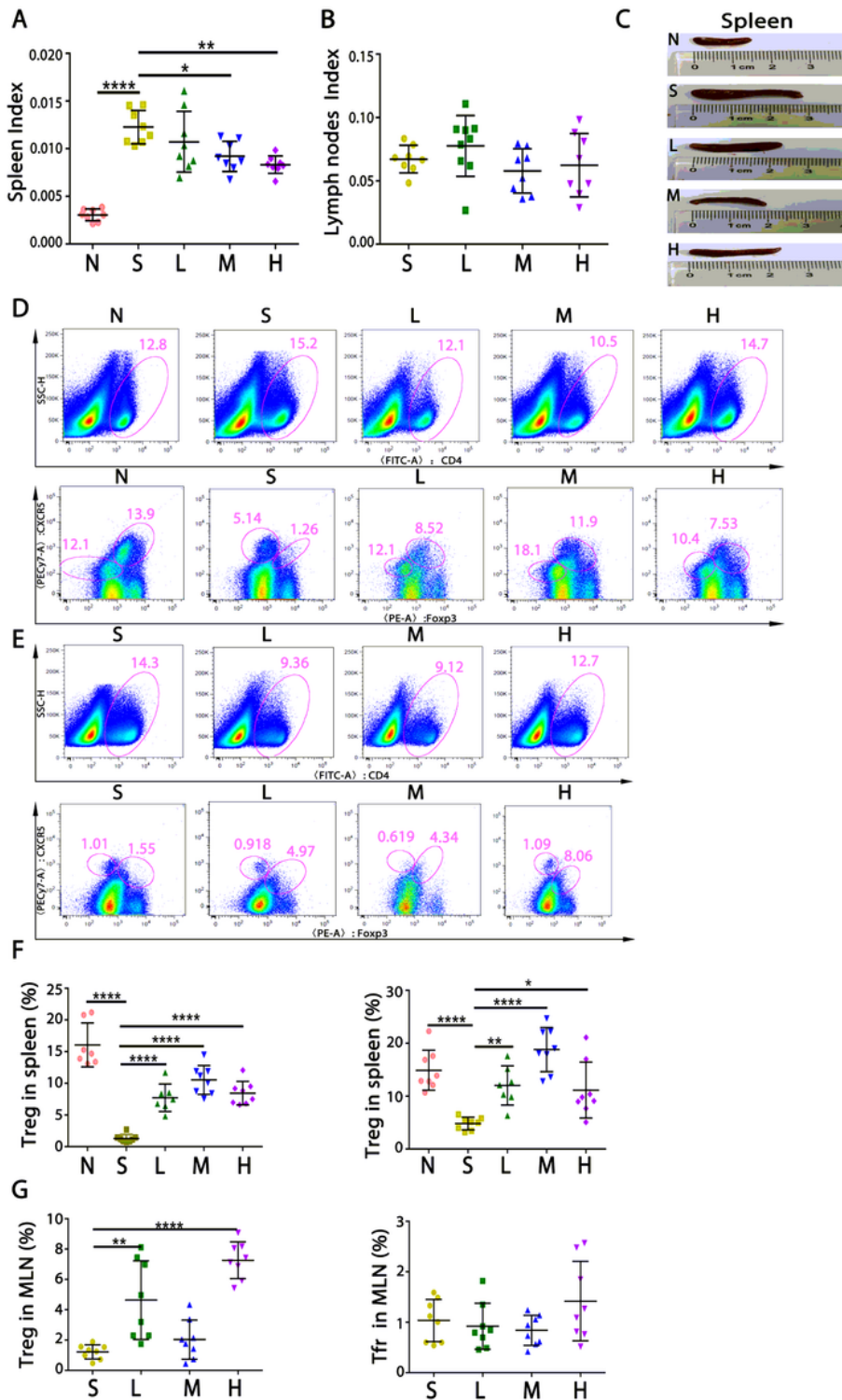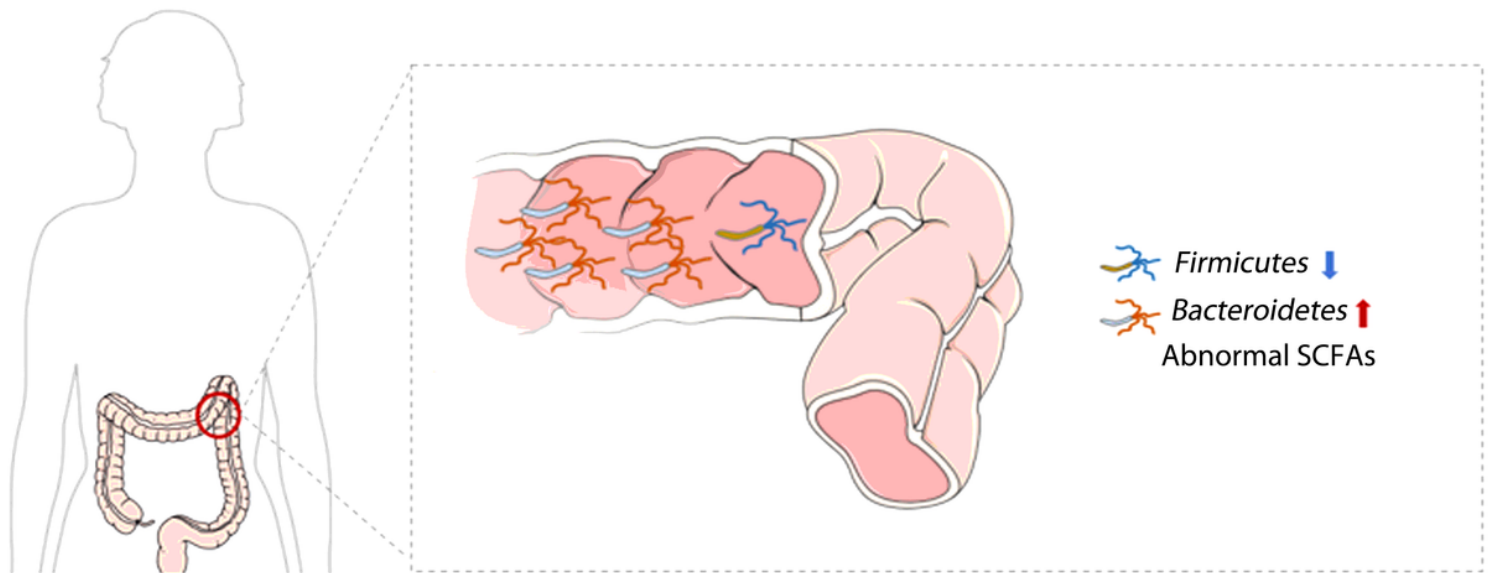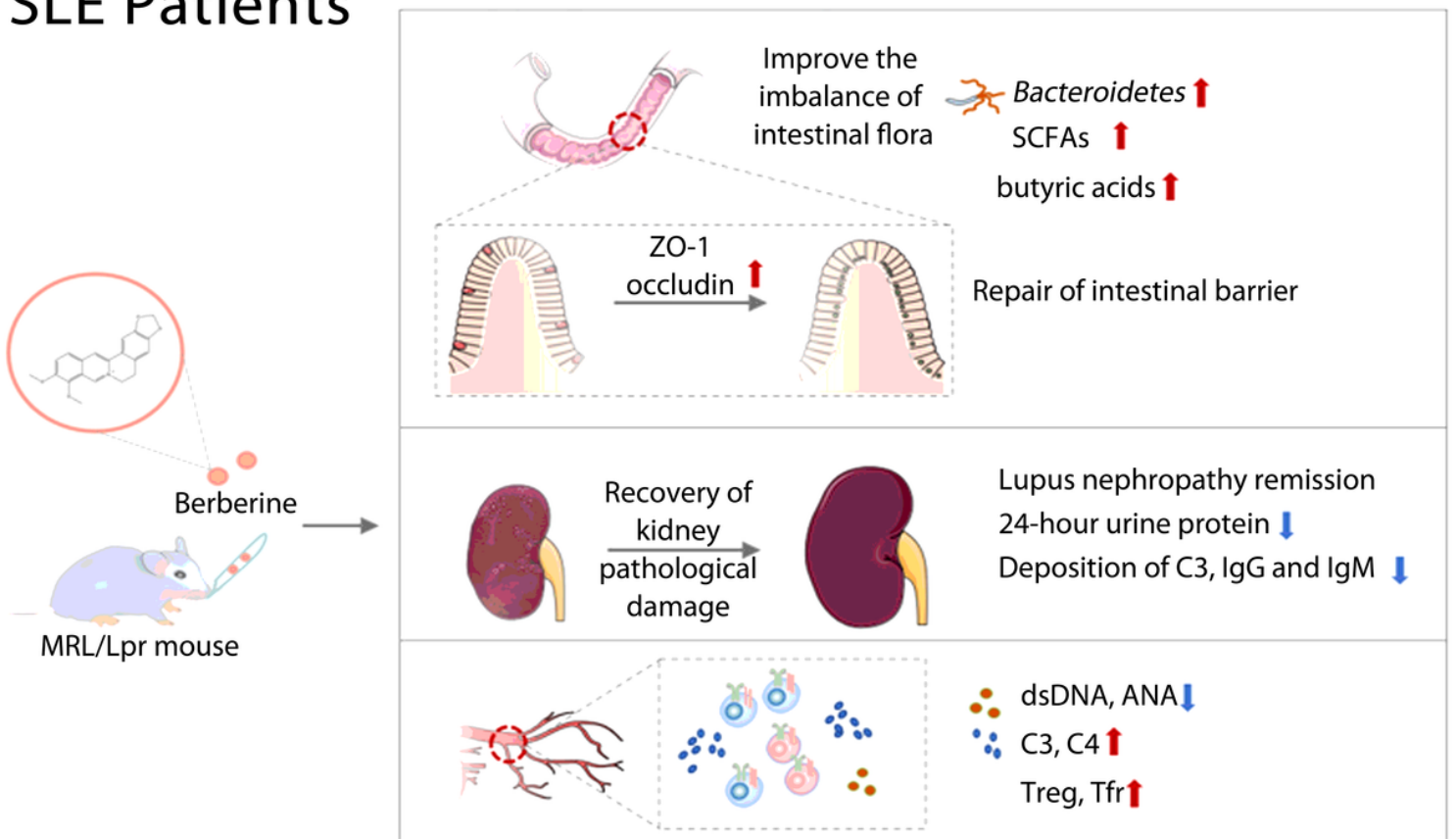

Figure 7

Berberine inhibits the abnormal activation of the immune system in MRL/Lpr mice via the activation of Treg and Tfr cells in spleen and MLN. (A) Spleen index: spleen weght to body weight in five groups of mice. n=8 in each group. $*p < 0.05, **p < 0.01, ****p < 0.0001$ by one-way ANOVA. (B) lymph nodes index: lymph nodes weght to body weight in five groups of mice. n=8 in each group. (C) Berberine treatment inhibited spleen enlargement. (D) Flow cytometry dot plots of Treg and Tfr cells in spleen of five groups of mice. n=8 in each group. (E)Flow cytometry dot plots of Treg and Tfr cells in MLN of four groups of mice. n=8 in each group. (F) Quantification of Treg (left) and Tfr (right) cells in spleen of five groups of mice. n=8 in each group.$*p < 0.05, **p < 0.01, ****p < 0.0001$ by one-way ANOVA. (G) Quantification of Treg (left) and Tfr (right) cells in MLN of four groups of mice. n=8 in each group. $**p < 0.01, ****p < 0.0001$ by one-way ANOVA.

Figure 8

Gut microbiota and metabolites were abnormal in Chinese SLE patients. The rapeutic effects of berberine in MRL/Lpr mice. Chinese SLE patients showed the increased Bacteroidetes and abnormal SCFAs in gut. Oral treatment of berberine changed the intestinal flora and SCFAs, enhanced the intestinal barrier, recovered the kidney and rugulated the immune system in MRL/Lpr mice. SLE, Systemic Lupus Erythematosus; SCFAs, short-chain-fatty acids; dsDNA, double- stranded deoxyribonucleic acid antibodies; ANA, antinuclear antibody; Treg, regulatory T cells; Tfr, follicular regulatory T cells.

Supplementary Files

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