Probiotics Improve Autoimmune Hepatitis via Gut Mycobiota-Mediated Follicular Helper T Cells

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Research

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Abstract

Background: Autoimmune hepatitis (AIH) is a chronic, immune-mediated liver dysfunction. The follicular helper (TFH) T cells play critical roles in the immunopathogenesis and progression of AIH. But the underlying mechanism of the dysregulation of TFH cells in AIH remains to be determined. Therefore, we aimed to investigate the effect of gut mycobiota on TFH cell response in AIH.

Methods: Samples from AIH patients and the EAH animal model were analyzed using Real-time quantitative PCR (RT-qPCR), Enzyme-linked immunosorbent assay (ELISA), western blotting, flow cytometry, and Hematoxylin-eosin (HE) staining to determine the role of gut mycobiota on autoimmune hepatitis.

Results: Lactobacillus capsule could significantly increased the levels of Bacteroides fragilis, Clostridium, Clostridium leptum, Bifi, and Lacto in AIH patients and significantly decreased the levels of ALT, AST, TBIL, SMA, ANA, DAO, and ET in AIH patients. What's more, the Lactobacillus capsule showed similar results in EAH mice. it decreased the levels of serum IL-21, the proportions of TFH cells in CD4+ T cells as well as TFH related cytokines and factors. Mechanistically, lactobacillus capsule regulated TFH response in EAH mice in DCs and MyD88/NF- κ B pathway-dependent manner.

Conclusion: Our results suggested a protective and therapeutic potential of probiotics in the treatment of AIH.

Background

Autoimmune hepatitis (AIH) is a chronic, immune-mediated liver dysfunction [1, 2]. It is characterized by increased hypergammaglobulinaemia, damaged hepatocytes, and positive circulating autoantibodies [2, 3]. Autoantibodies are responsible for the pathogenesis of AIH, which decorate hepatocytes and induce complement-mediated cell lysis [4]. It is generally believed that autoantibodies production by the germinal center (GC) B cells requires the follicular helper (TFH) T cells [5, 6]. TFH cells are a specialized subset of CD4 + T cells that interact with B cells and are essential for B-cell activation, survival, and differentiation [6]. B cells' activation, survival, and differentiation require the secretion of soluble cytokines interleukin-4 (IL-4) and IL-21 from TFH cells [7]. We have previously reported that there was an increased number of circulating TFH cells and elevated serum IL-21 in AIH patients and experimental autoimmune hepatitis (EAH) mice model [8]. Therefore, TFH cells could be an essential regulator in humoral immune responses during AIH progression. However, the underlying mechanism of TFH cells' dysregulation in AIH remains to be determined.

Recently, the Gut-Liver Axis is widely practicable in the clinical settings of numerous diseases [9, 10]; thus, the role of gut microbiota in the regulation of AIH has received ample attention [1, 11]. Yiran et al. reported altered gut microbiome composition and function in AIH, potentially wielding gut microbiota as noninvasive biomarkers to assess disease activity [1]. Moreover, the data from humanized murine models showed that gut microbiota is engaged in the pathogenesis of AIH [12]. Various reports have elucidated

the relationship between gut microbiota and TFH cells. Gut microbiota has been reported to regulate arthritis, an autoimmune disease, through TFH but not Th17 cells [13]. Teng et al. showed that Peyer's patch TFH cells were essential for gut commensal segmented filamentous bacteria (SFB)-induced systemic arthritis despite the production of auto-antibodies predominantly occurring in systemic lymphoid tissues [14]. Given the vital role of TFH cells in the pathogenesis of AIH, questions have emerged concerning the effect of gut microbiota on TFH cells in the pathogenesis of AIH.

Probiotics are living microbiota, and their administration conferred health benefits to the host by altering the gut microbiota composition [15]. VSL#3, a mixture of 8 probiotic bacteria, was able to alleviate dextran sulfate sodium-induced colitis by downregulating TFH cells [16]. Arai et al. showed that orally administered probiotic *Lactobacillus paracasei* MCC1849 increased the proportion of IgA + B cells and TFH cells in Peyer's patches in mice [17]. In this study, we investigated the effect of lactobacillus capsules on TFH cell response in AIH. Lactobacillus capsule improved the symptoms of AIH patients and EAH mice by inhibiting TFH cell response and regulating the dendritic cells (DC) via the TLR4/MyD88/NF- κ B pathway.

Materials And Methods

Participants and treatment

Fifty patients with active AIH were recruited at The First People's Hospital of Changzhou. All patients were diagnosed according to the AIH simplified diagnostic score system proposed by the International Immune Hepatitis Group (IAIHG) in 2008 and the AIH diagnosis and treatment guidelines updated by the American Association for the Study of Liver Diseases (AASLD) in 2010. The experimental protocol conformed to the 1975 Declaration of Helsinki (6th revision, 2008) and is approved by the Ethical Committee of The First People's Hospital of Changzhou and the Third Affiliated Hospital Soochow University. Informed consent was obtained from each participant.

All patients were randomly divided into the following groups: group I, prednisone treatment, and group II, prednisone + lactobacillus capsule treatment. On days 3 and 7 after treatment, patients' fecal and blood plasma were collected and subjected to gut microbiota and clinical index analysis.

Establishment of experimental autoimmune hepatitis (EAH) model

Female C57BL/6 mice (6-8 weeks old) were purchased from Nanjing Experimental Animal Center (Jiangsu, China). CD103-deficient (CD103^{-/-}) and MyD88-deficient (MyD88^{-/-}) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). All experiments involved in animal was approved by the Ethical Committee of The First People's Hospital of Changzhou and the Third Affiliated Hospital of Soochow University.

The EAH model was established as described previously [8, 18]. The effect of lactobacillus capsule treatment on EAH was determined in model mice; the mice were randomly divided into the following

groups: group I, sham controls; group II, prednisone (0.5g/kg/day) treatment and group III, prednisone (0.5g/kg/day)+ lactobacillus capsule (0.66g/day) treatment. On day seven after treatment, the fecal, blood plasma, liver tissue, and colon tissue of model mice were collected.

The effect of DCs and MyD88 in gut microbiota-mediated TFH response in EAH mice was determined in CD103^{-/-} or MyD88^{-/-} mice to mimic the EAH model, then the mice were treated with prednisone (0.5g/kg/day) or prednisone (0.5g/kg/day)+ lactobacillus capsule (0.66g/day).

Real-time quantitative PCR (RT-qPCR) for 16S rRNA

Total DNA from fecal pellets of AIH patients and mice was isolated using the QIAamp Fast DNA stool mini kit (Qiagen) following the manufacturer's protocol. RT-qPCR primers utilized in the present experiments was as follows: *Bacteroides fragilis*, forward (5'-3') TTCAACCTGATCGATCCGGAAGATCCG, reverse (5'-3') GCTGGTAGACTACCTGAGTAAGGAGTC; *Clostridium* forward (5'-3') TTGAGCGATTTACTTCGGTAAAGA, reverse (5'-3') TGTACTGGCTCACCTTTGATATTCA; *Clostridium leptum* forward (5'-3') GCACAAGCAGTGGAGT, reverse (5'-3') CTTCTCCGTTTTGTCAA; *Bifi* forward (5'-3') TCTGGCTCAGGATGAACGC, reverse (5'-3') CACCGTTACACGGGAATTC; *Lacto* forward (5'-3') TGGAAACAGRTGCTAATACCG, reverse (5'-3') GTCCATTGTGGAAGATTCCC. Cycling conditions were 95°C for 40 s; 40 cycles of 95°C for 5 s, 60°C for 30 s; 95°C for 15 s; and 60°C for 1 h.

Clinical index assay

The level of alanine transaminase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), albumin, smooth muscle antibody (ANA/SMA), IgG, IgA, and IgM in serum were determined using the corresponding implements according to the manufacturers' instruction (Beckman Coulter).

Enzyme-linked immunosorbent assay (ELISA) assay

The endotoxin (ET), diamine oxidase (DAO), TGF- β , IL-10, IL-6, and TNF- α ELISA kits were obtained from DECO. The KGF-1 and KGF-1 ELISA kits were obtained from Abcam. The IL-21 ELISA kits were obtained from Multisciences. The levels of ET, DAO, TGF- β , KGF-2, KGF-1, IL-10, IL-6, IL-21, and TNF- α were measured according to the protocol of ELISA assay kits.

Hematoxylin-eosin (HE) staining

Liver and colon tissues were collected from the EAH model for HE staining. HE staining was performed according to the previous report [18].

Flow cytometric analysis

Peripheral blood mononuclear cells (PBMCs) from AIH patients and EAH mice were isolated by density gradient centrifugation according to the manufacturer's instruction. The TFH cells in PBMC were stained with BV510-anti-CD4 (0.2 mg/mL), PerCP-Cy5.5-anti-CXCR5 (0.1 mg/mL), and PE-anti-FoxP3 (0.1 mg/mL) as previously described [8, 18].

RT-qPCR assay

Total RNA from peripheral blood mononuclear cells (PBMCs) or tissue was extracted using TRIzol reagent (TaKaRa) according to the manufacturers' instructions. A cDNA Reverse Transcription Kit (TaKaRa) and an SYBR PrimeScript RT-qPCR Kit (Takara) were used for mRNA expression assessment according to the manufacturer's instructions. The primers' sequences were listed in Table 1. GAPDH served as an internal reference.

Western Blot assay

Total protein from PBMCs was extracted using RIPA lysis buffer containing phosphatase and protease inhibitors (Beyotime Biotech). Total protein (30 µg) from each sample was separated on a 10% SDS PAGE gel as previously described [19]. The antibodies included anti-TLR4 (CST), anti-MyD88 (CST), anti-p65 (Abcam), anti-p-p65 (Abcam) and anti-ACTB (CST).

Statistical analysis

The student's t-test was used to assess statistical significance among different experimental groups. Data are expressed as the mean ± SEM. *P < 0.05 and **P < 0.01 levels were considered significant.

Results

Lactobacillus capsules improved the clinical symptoms of patients with AIH.

Patients received prednisone or prednisone + lactobacillus capsules to determine the effect of probiotic treatment on AIH progression. As shown in Fig. 1A, the levels of probiotics *Bacteroides fragilis*, *Clostridium*, *Clostridium leptum*, *Bifi*, and *Lacto* were significantly higher in the fecal pellets of AIH patients who received prednisone + lactobacillus capsule treatment than that of AIH patients who received prednisone. Serological test results revealed that prednisone treatment significantly decreased the levels of ALT, AST, TBIL, SMA, and ANA; while prednisone + lactobacillus capsule treatment further reduced the expression of ALT, AST, TBIL, SMA, and ANA in the serum of AIH patients (Fig. 1B). Moreover, prednisone + lactobacillus capsules administration has markedly downregulated serum DAO and ET levels in AIH patients compared with patients who received prednisone treatment (Fig. 1C, D).

Lactobacillus capsule ameliorates EAH

We further used the EAH model to demonstrate the effect of probiotic treatment on AIH progression. When EAH model mice were administered prednisone + lactobacillus capsules, the levels of probiotics *Bacteroides fragilis*, *Clostridium*, *Clostridium leptum*, *Bifi*, and *Lacto* were significantly increased in the fecal pellets (Fig. 2A). Lactobacillus capsule treatment improved the intestinal barrier of EAH mice (Fig. 2B). Moreover, lactobacillus capsule treatment significantly reduced ALT, AST, TBIL, DAO, and ET levels and attenuate liver injury of EAH mice (Fig. 2C-E).

Lactobacillus capsule inhibited TFH response and regulated cytokines expression in EAH mice

Our previous studies have indicated that the uncontrolled accumulation of TFH cells plays an important role in the pathogenesis of AIH [8, 18]. As shown in Fig. 3A, prednisone + lactobacillus capsule treatment has markedly decreased the levels of serum IL-21 in EAH mice. In accordance with decreased IL-21 levels in the serum, the proportions of TFH cells in CD4⁺ T cells were significantly downregulated in the blood of EAH mice after prednisone + lactobacillus capsule treatment (Fig. 3B). Moreover, the TFH-related mRNA expression levels of IL-21, Bcl-6, and CXCR5 were substantially decreased in the liver of EAH mice after prednisone + lactobacillus capsule treatment (Fig. 3C).

Next, we evaluated the levels of six different cytokines in the serum of EAH mice treated with prednisone + lactobacillus capsule. TGF- β , KGF-1, KGF-2, and IL-10 were decreased in EAH mice compared to the control group (Figure 4). However, prednisone + lactobacillus capsule treatment promoted the expression of TGF- β , KGF-1, KGF-2, and IL-10. Furthermore, IL-6 and TNF- α were upregulated in EAH mice, and prednisone + lactobacillus capsule treatment markedly decreased their expression (Fig. 4).

Lactobacillus capsules regulated TFH response in EAH mice through DC cells.

Previous studies showed that gut microbiota regulates T cell activation and differentiation in DC dependent manner [20]. Herein, we hypothesized that lactobacillus capsule inhibited TFH response in EAH mice by regulating DC cells. CD103^{-/-} mice were used to establish the EAH model. As shown in Fig. 5A, the serum IL-21 in CD103^{-/-} mice was significantly lower than in WT mice after EAH induction. Prednisone + lactobacillus capsule treatment downregulated the levels of IL-21 in WT mice without affecting the IL-21 expression in CD103^{-/-} mice after EAH induction. More importantly, prednisone + lactobacillus capsule treatment reduced the proportions of TFH cells in CD4⁺ T cells of WT mice but did not influence the proportions of TFH cells in CD103^{-/-} mice after EAH induction (Fig. 5B). Also, CD103 knockout reversed the effect of prednisone + lactobacillus capsule treatment on cytokines expression in EAH mice (Fig. 5C).

Lactobacillus capsule regulated TFH response in EAH mice via TLR4/MyD88/NF- κ B pathway

Given that the TLR4/MyD88/NF- κ B pathway is a key regulator for gut microbiota-mediated DC activation, we explored whether the Lactobacillus capsule regulates TFH response in EAH mice concerning this pathway. As shown in Figure 6A, the mRNA levels of TLR4 and MyD88 were significantly increased in EAH mice. However, prednisone + lactobacillus capsule treatment reduced TLR4 and MyD88 mRNA expression (Fig. 6A). Furthermore, The upregulated TLR4, MyD88, and p-p65 proteins in EAH mice were downregulated after the treatment with prednisone + lactobacillus capsules (Fig. 6B).

Next, we used MyD88^{-/-} mice to further investigate the effect of the TLR4/MyD88/NF- κ B pathway on gut microbiota-mediated TFH response. As shown in Figure 7A, the serum IL-21 in MyD88^{-/-} mice was significantly lower than that in WT mice after EAH induction. Prednisone + lactobacillus capsule treatment downregulated the levels of IL-21 in WT mice but did not affect IL-21 expression in MyD88^{-/-}

mice after EAH induction (Fig. 7A). More importantly, Prednisone + lactobacillus capsule treatment reduced the proportions of TFH cells in CD4+ T cells of WT mice but did not influence the proportions of TFH cells in MyD88 ^{-/-} mice after EAH induction (Fig. 7B). Also, MyD88 knockout reversed the effect of prednisone + lactobacillus capsule treatment on cytokines expression in EAH mice (Fig. 7C).

Discussion

In the last decade, an overwhelming amount of evidence indicated that gut microbiota is a key mediator of autoimmune diseases. In Albino Oxford rats, gut microbiota modulation ameliorated the experimental autoimmune encephalomyelitis, a chronic inflammatory disease of the central nervous system, by regulating IFN- γ and IL-17 production [21]. Gut microbiota and bacterial translocation promoted autoimmune cholangitis by TLR2-independently increased gut permeability [22]. In AIH patients, the distinct microbial composition is associated with disease progression. Notably, the most strongly disease-associated taxa were positively correlated with the serum level of aspartate aminotransferase and liver inflammation [1]. Cai et al. used transgenic AIH mice carrying HLA-DR3 and showed a close relationship between microbiota and AIH [11]. In the current study, we demonstrated that lactobacillus capsules increased the levels of *Bacteroides fragilis*, *Clostridium*, *Clostridium leptum*, *Bifi*, and *Lacto* in AIH patients, significantly decreased the levels of ALT, AST, TBIL, SMA, ANA, DAO, and ET in the serum of patients with AIH. Moreover, similar results were found in EAH mice. Hence, altering gut microbial composition by lactobacillus capsule may improve the disease in patients and model mice.

Previous studies have reported the crucial roles of TFH cells in autoimmune diseases [23]. Alcohol and its metabolite acetate promote autoimmune arthritis by altering the functional state of TFH cells in vitro and in vivo [24]. Faliti et al showed that P2X7 receptor restrained pathogenic TFH cell generation in systemic lupus erythematosus [25]. Also, our team has previously reported the importance of TFH in AIH patients and EAH mice [8, 18]. Dysregulation between TFR and TFH cells caused excessive autoantibodies production and immune homeostasis destruction, leading to the immunopathological process in AIH patients [8]. Moreover, we have explored the significance of TFH and related molecules on C57BL/6 mice with EAH [18]. Gut microbiota has been reported to regulate the biological functions of TFH cells in autoimmune diseases [13, 14]. Therefore, we explored how gut microbiota affects TFH cells in the pathological process of AIH. Our results showed that lactobacillus capsules decreased the levels of serum IL-21 as well as the proportions of TFH cells in CD4 + T cells in EAH mice. Moreover, lactobacillus capsule affected the expression of TFH related cytokines and factors including IL-21, Bcl-6, CXCR5, TGF- β , KGF-1, KGF-2, IL-10, IL-6 and TNF- α . These results indicated that lactobacillus capsule may inhibit TFH response and regulate cytokines expression in EAH mice.

It is well known that gut microbiota could regulate T cell activation and differentiation in DC dependent manner [20]. For example, gut microbiota modulates Mincle-Syk Axis in DCs, and regulates the production of IL-17 and IL-22 by CD4 T cells, promoting intestinal barrier integrity [26]. Herein, we hypothesized that lactobacillus capsule inhibited TFH response in EAH mice by regulating DC cells. We used CD103 ^{-/-} mice and found that lactobacillus capsule decreased the levels of serum IL-21, the proportions of TFH cells in

CD4 + T cells as well as TFH related cytokines in WT mice, but had no influence on the TFH response in CD103 ^{-/-} mice after EAH induction. These results indicated that lactobacillus capsules might inhibit TFH response in EAH mice via DCs.

Intestinal DCs could recognize gut microbiota through TLRs, leading to the activation of the MyD88/NF- κ B pathway and regulation of T cell activation and differentiation. Liang et al. showed that functionally specialized DCs promoted inflammatory Th17 via MyD88 [27]. Therefore, we investigated if the lactobacillus capsule regulated TFH response in EAH mice via MyD88/NF- κ B pathway. Our results indicated that lactobacillus capsule treatment downregulated both the mRNA and protein levels of TLR4, MyD88, and p-p65 in EAH mice. More importantly, we found that lactobacillus capsule decreased serum IL-21, the proportions of TFH cells in CD4 + T cells, and TFH related cytokines in WT mice but had no influence on the TFH response in MyD88 ^{-/-} mice after EAH induction. These results indicated that lactobacillus capsules might inhibit TFH response in EAH mice via the TLR4/MyD88/NF- κ B pathway. However, our study possesses some limitations; we could not investigate distinct critical signaling pathways in DCs, such as the TRAM/TRIF pathway, and their role in the TFH response in AIH [28]. Further in-depth research is still needed.

Conclusion

In summary, our results showed that lactobacillus capsules could improve AIH in humans and mice by altering gut microbial composition and inhibiting TFH response. Furthermore, we also demonstrated that lactobacillus capsules treatment regulated TFH response in EAH mice in regard to DCs and via MyD88/NF- κ B pathway. Therefore, our results suggest a protective and therapeutic potential of lactobacillus capsules in the treatment of AIH.

Abbreviations

AIH, Autoimmune hepatitis;

TFH, The follicular helper;

GC, Germinal center;

IL-4, Interleukin-4;

EAH, Experimental autoimmune hepatitis;

SFB, segmented filamentous bacteria;

IAIHG, International Immune Hepatitis Group;

AASLD, American Association for the Study of Liver Diseases;

ALT, Alanine transaminase;

AST, Aspartate aminotransferase;

TBIL, Total bilirubin;

SMA, Smooth muscle antibody;

ET, Endotoxin;

DAO, Diamine oxidase;

HE, Hematoxylin-eosin;

RT-qPCR, Real-time quantitative PCR;

ELISA, Enzyme-linked immunosorbent assay;

PBMCs, Peripheral blood mononuclear cells

Declarations

Ethics approval and consent to participate

All experimental procedures involving animals have followed the standard ethical guidelines of the Ethical Committee of The First People's Hospital of Changzhou and the Third Affiliated Hospital of Soochow University. All efforts were made to ensure mere animal suffering and to minimize the number of mice used.

Consent for publication

The manuscript has been reviewed and approved by all authors and represents original work that has not been published elsewhere and is not under consideration for publication elsewhere.

Availability of data and material

All data collection and analysis were conducted under double blind and were supported by The First People's Hospital of Changzhou and the Third Affiliated Hospital of Soochow University. We will provide the original data at any time if necessary.

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

Conceptualization: Liang Ma, Liwen Zhang, Yun Zhuang, Yanbo Ding, Jianping Chen;

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Formal analysis: Liang Ma, Liwen Zhang, Yun Zhuang, Yanbo Ding;

Funding acquisition: Jianping Chen;

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Methodology: Liang Ma, Liwen Zhang, Yun Zhuang, Yanbo Ding;

Project administration: Jianping Chen;

Resources: Jianping Chen;

Software: Jianping Chen;

Supervision: Jianping Chen;

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Roles/Writing - original draft: Liang Ma, Liwen Zhang, Yun Zhuang, Yanbo Ding;

Writing - review & editing: Liang Ma, Liwen Zhang, Jianping Chen.

All authors read and approved the final manuscript.

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Tables

Table 1 The RT-qPCR primers used in this study

Primer Name	Primer Sequence (5'-3')
Bcl-6 Forward	CGCATGTGTGGCATCAACG
Bcl-6 Reverse	TCCCAACATAGTCCATTTTTTGGC
CXCR5 Forward	TGGCCTTCTACAGTAACAGCA
CXCR5 Reverse	GCATGAATACCGCCTTAAAGGAC
IL-21 Forward	ATTAAAGCTTCTGGTGGCATGGAGAGGAC
IL-21 Reverse	TAGGATCCTGTGTTCTAGGAGAGATGCTG
MyD88 Forward	TCATGTTCTCCATACCCTTGGT
MyD88 Reverse	AAACTGCGAGTGGGGTCAG
TLR4 Forward	AGCTCCTGACCTTGGTCTTG
TLR4 Reverse	CGCAGGGGAACCTCAATGAGG
GAPDH Forward	AATGGATTTGGACGCATTGGT
GAPDH Reverse	TTTGCACTGGTACGTGTTGAT

Figures

A

Relative levels	Prednisone			Prednisone+Lactobacillus capsule		
	0 day	3 days	7 days	0 day	3 days	7 days
Bacteroides fragilis	1±0.1468	1.2342±0.2314	0.94321±0.2415	1.3233±0.1326	4.3863±0.4532**	10.06951±1.3742**
Clostridium	1±0.2534	0.94371±0.1167	0.9365±0.2387	0.9175±0.2147	3.72551±0.4337**	8.0845±1.3829**
Clostridium leptum	1±0.1354	1.21881±0.3145	1.2752±0.3352	1.1069±0.1582	3.5321±0.3651**	6.9934±1.4537**
Bifi	1±0.1983	1.0345±0.3627	1.28371±0.2004	0.9877±0.1633	4.0365±0.6721**	8.6533±1.5332**
Lacto	1±0.1168	1.3866±0.2148	1.4531±0.1988	1.1023±0.1542	5.9836±1.1023**	15.7761±0.8795**

B

Relative levels	Prednisone		Prednisone+Lactobacillus capsule	
	0 day	7 days	0 day	7 days
ALT	1±0.2342	0.4942±0.1823*	1.0243±0.1234	0.2142±0.0232**
AST	1±0.3273	0.5321±0.2753*	0.89241±0.0941	0.3422±0.0621**
TBIL	1±0.0322	0.6023±0.0232*	1.0912±0.1823	0.5321±0.1432**
SMA	1±0.2032	0.4531±0.1892**	1.0913±0.1231	0.3242±0.1643**
ANA	1±0.1115	0.3531±0.1123**	0.9623±0.1865	0.3012±0.1240**

Figure 1

Lactobacillus capsule improves the clinical symptoms of AIH patients. (A) The levels of *Bacteroides fragilis*, *Clostridium*, *Clostridium leptum*, *Bifi*, and *Lacto* in the fecal pellets of AIH patients received prednisone or prednisone + lactobacillus capsule. (B) The levels of ALT, AST, TBIL, SMA, and ANA in the serum of AIH patients received prednisone or prednisone + lactobacillus capsule. (C, D) The level of DAO (C) and ET (D) in the serum of AIH patients received prednisone or prednisone + lactobacillus capsule. The data are presented as the mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$.

Figure 2

Lactobacillus capsule ameliorates EAH in mice. (A) The levels of *Bacteroides fragilis*, *Clostridium*, *Clostridium leptum*, *Bifi*, and *Lacto* in the fecal pellets of EAH mice received prednisone or prednisone + lactobacillus capsule. (B) Representative histological picture of the colon in animals after prednisone or prednisone + lactobacillus capsule (magnification, 200 $\times$). (C) The levels of ALT, AST, TBIL, SMA, and ANA in the serum of EAH mice received prednisone or prednisone + lactobacillus capsule. (D) The level of DAO and ET in the serum of EAH mice received prednisone or prednisone + lactobacillus capsule. (E) Representative histological picture of the liver in animals after prednisone or prednisone + lactobacillus capsule (magnification, 200 $\times$). The data are presented as the mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$.

Figure 3

Lactobacillus capsule inhibits TFH response in EAH mice. (A) The level of IL-21 in the serum of EAH mice received prednisone or prednisone + lactobacillus capsule. (B) The portion of TFH cells in CD4⁺ T cells in the PBMcs of EAH mice received prednisone or prednisone + lactobacillus capsule. (C) The mRNA levels of IL-21, Bcl-6, and CXCR5 in the liver of EAH mice received prednisone or prednisone + lactobacillus capsule. The data are presented as the mean $\pm$ SEM. *P<0.05.

Figure 4

Lactobacillus capsule regulated cytokines expression in EAH mice. The protein levels of TGF-β, KGF-1, KGF-2, IL-10, IL-21, IL-6, and TNF-α in the serum of EAH mice received prednisone or prednisone + lactobacillus capsule. The data are presented as the mean ± SEM. *P<0.05.

Figure 5

Lactobacillus capsule inhibits TFH response in EAH mice through DC cells. (A) The level of IL-21 in the serum of WT or CD103^{-/-} EAH mice received prednisone or prednisone + lactobacillus capsule. (B) The portion of TFH cells in CD4⁺ T cells in the PBMCs of WT or CD103^{-/-} EAH mice received prednisone or prednisone + lactobacillus capsule. (C) The protein levels of IL-6, IL-10, KGF-1, and KGF-2 in the serum of WT or CD103^{-/-} EAH mice received prednisone or prednisone + lactobacillus capsule. The data are presented as the mean $\pm$ SEM. *P<0.05.

Figure 6

Lactobacillus capsule inhibits the expression of TLR4/MyD88/NF- κ B pathway-related genes. (A) The mRNA levels of TLR4 and MyD88 in EAH mice received prednisone or prednisone + lactobacillus capsule. (B) The protein levels of TLR4, MyD88, p65, and p-p65 in EAH mice received prednisone or prednisone + lactobacillus capsule. The data are presented as the mean $\pm$ SEM. * $P < 0.05$.

Figure 7

Lactobacillus capsule inhibits TFH response in EAH mice through MyD88. (A) The level of IL-21 in the serum of WT or MyD88^{-/-} EAH mice received prednisone or prednisone + lactobacillus capsule. (B) The portion of TFH cells in CD4⁺ T cells in the PBMCs of WT or MyD88^{-/-} EAH mice received prednisone or prednisone + lactobacillus capsule. (C) The protein levels of IL-6, IL-10, KGF-1, and KGF-2 in the serum of WT or MyD88^{-/-} EAH mice received prednisone or prednisone + lactobacillus capsule. The data are presented as the mean $\pm$ SEM. *P<0.05.