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Lactobacillus rhamnosus GG alleviates β -conglycinin-induced allergy by regulating the T cell receptor signaling pathway†

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Currently, the need for safe and effective methods for relieving allergies is an important concern. In this study, we evaluated the role of *Lactobacillus rhamnosus* GG (LGG) in alleviating β -conglycinin (β -CG)-induced allergies and elucidated the related molecular mechanisms. Typical allergy symptoms and inflammatory factors in the serum showed that LGG intervention effectively alleviated β -CG induced allergy in mice, which was better than natural recovery (NR). Intestinal villi were restored and lower levels of CD4+ T cells infiltrated after LGG intervention. We evaluated whether LGG intervention weakened the proliferation ability of the spleen cells of allergic mice, balancing between T/B cells and Th1/Th2 and Th17/Treg cytokines. Transcriptome analysis revealed that 4106 differentially expressed mRNAs were identified by comparing the LGG group and β -CG group, and 546 differentially expressed mRNAs were identified by comparing the LGG group and NR group. KEGG pathway analysis identified that the T cell receptor (TCR) signaling pathway was significantly enriched upon LGG intervention, and the upregulated *Ifnar2* and the downregulated *Tgfb2*, *Il13r2* and *Il4ra* were further validated by qPCR analysis. Therefore, the above results fully revealed the important role of LGG in alleviating β -CG-induced allergies.

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1. Introduction

With the increase in the prevalence of food allergies, global attention to food safety and public health issues has attracted increased attention.^{1–3} Food allergies can affect adults and children significantly by 3–4% and 6–8%, respectively.⁴ Most food allergies are caused by proteins from peanuts, soy, milk, eggs, fish, shellfish and nuts,⁵ which are considered the eight food allergens by the Codex Food Labeling Committee.

Currently, at least 16 kinds of sensitization proteins detected in soybean have been reported to have binding properties with serum immunoglobulin E (IgE) from soybean allergy patients, among which β -conglycinin (β -CG) accounts for approximately 30% of the total soybean proteins⁶ and is treated as the main allergen.⁷ β -CG has a trimer structure that consists of an α -subunit, α' -subunit and β -subunits,⁸ with a stable space structure, and the sensitization is relatively strong enough and not easily destroyed by food processing.

Therefore, effective relief of the related allergy has become an important issue that needs to be addressed.

It has been reported that probiotics could effectively relieve the symptoms caused by allergies.⁹ Probiotics are a class of active microorganisms that are beneficial to the host. Probiotics can colonize the human intestine to improve the balance of the microecology of the host and play a beneficial role in altering the composition of the gut microbes and the host's immune response.^{10,11} It has been reported that probiotics can participate in the regulation of cytokines and regulatory T cells (Tregs).¹² As one of the most accepted probiotic, LGG has been reported to have the functionality to prevent or alleviate certain allergic symptoms.¹³ LGG has been reported to reduce Th1 differentiation of interferon- γ (IFN- γ) through immune regulation to alleviate milk allergies,¹⁴ increase infant tolerance to milk allergies by regulating the balance of Th1/Th2,¹⁵ and restore intestinal homeostasis and prevent or alleviate allergies by interacting with intestinal immune cells.¹⁶ The intervention effect of LGG on cow's milk protein-induced allergy may rely on the attributable gut homeostasis restoration by ameliorating the gut environment in infants.¹⁷ LGG can improve the allergic tolerance to cow's milk and affect the abundance of the bacterial community in infants.¹⁸ LGG has an immunostimulatory effect on the saliva mucosa, which is shown as increased levels of rBet v1- and rMal d1-specific IgA in saliva.¹⁹

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Investigation of the mechanism of relieving food allergies is the basis of developing effective methods for prevention or curing allergies. At present, transcriptome analysis is used to reveal the molecular mechanism. Through transcriptome analysis and qPCR data, ipratropium bromide treatment was verified to regulate the expression of allergic immunity-related mRNAs and miRNAs in the nasal mucosa of allergic mice, which may be related to the reduction of nasal allergy.²⁰ Transcriptomics of sputum cells from asthma patients determined the differentially expressed genes *TAC1*, *TAC2* and *TAC3* between eosinophil and non-eosinophil-related sputum inflammation, characterized by inflammasome-associated and metabolic mitochondrial pathways.²¹ Key genes, biological processes, and cell types related to peanut allergy were analysed through transcriptomics.²²

At present, there are no reports of dietary interventions for soybean allergies. Therefore, the main purpose of this research is to use transcriptome analysis to study the role of LGG in alleviating β -CG-induced allergies and its underlying mechanisms, including the effect of LGG on T cell differentiation, especially the related signaling pathways. The results are expected to benefit the development of a practical strategy for LGG to alleviate allergies.

2. Materials and methods

2.1. Allergen and LGG

Soybean protein was extracted from soybean meal, and β -CG was isolated from soybean protein and purified by size exclusion chromatography (Superdex™ 200 Increase, 10/300 GL, GE General Electric Company) according to a previously reported method.²³

First, 0.1 g of freeze-dried LGG powder (Chr Hansen A/S, Denmark) was dissolved in 10 mL of Rogosa and Sharp (MRS) broth and incubated for 24 h at 37 °C. For oral gavage, the cultured LGG was centrifuged at 3000 rpm for 10 min, washed twice with saline, and resuspended to a final concentration of 1×10^9 colony-forming units (CFU) mL⁻¹.²⁴

2.2. Mice

Female BALB/c mice (4 weeks old) weighing 20–25 g were purchased from SPF (Beijing) Biotechnology Co., Ltd. The mice were fostered under standard conditions at a certain temperature (22 ± 2 °C) and humidity (70%) at the Institute of Radiation Medicine Chinese Academy of Medical Sciences. All mice were fed a normal diet for one week. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals and the experiments were approved by the Animal Ethics Committee of Institute of Radiation Medicine, Chinese Academy of Medical Sciences (IRM-DWLL-2020094).

2.3. Experimental design

2.3.1. Establishment of the β -CG-induced allergy mouse model. The mice were gavaged with a 300 μ L mixture of 1 mg

mL⁻¹ β -CG with 0.01 mg mL⁻¹ cholera toxin (CT) (Sigma-Aldrich, Israel, USA) once a week. On the fifth gavage, mice were gavaged with 300 μ L of a mixture of 2 mg mL⁻¹ β -CG together with 0.01 mg mL⁻¹ CT (named the β -CG group). The control group mice were gavaged with 300 μ L of CT and the saline group mice were gavaged with 300 μ L of saline. We collected blood from all the mice on the 7th, 14th, 21st, 28th, and 35th days for histamine content detection and allergy symptom scoring.

2.3.2. LGG intervention. The allergic mice were divided into two groups ($n = 12$ in each group) for LGG intervention and natural recovery. The mice for LGG intervention were gavaged with 300 μ L of LGG (1×10^9 CFU mL⁻¹ day⁻¹). After 6 days of intervention, half of the mice were sacrificed (named the LGG-1 group), and the other mice were sacrificed after 9 days of intervention (named the LGG-2 group). The naturally recovered mice served as the intervention control in which the allergic mice did not receive any treatment and were sacrificed at the same time as the LGG-1 group (named the NR-1 group) and the same time as the LGG-2 group (named the NR-2 group).

After sacrificing the mice, the blood and intestinal contents were collected and stored at -80 °C until further analysis. Fresh spleen cells were prepared from the extracted spleens for the following flow cytometry analysis to determine the differentiation of focused immune cells; moreover, rest of the spleen cells were stocked at -80 °C until further analysis of cytokines. RNA was extracted from the spleen for the following real-time quantitative polymerase chain reaction (qPCR) analysis and transcriptome sequencing analysis. The duodenum was collected and fixed in 4% paraformaldehyde for immunohistochemistry (IHC) analysis.

2.4. Allergy symptom score

The behaviour and physical characteristics of the mice were carefully observed for 30–50 min after challenge and assessed by symptom scores: 0-no abnormalities; 1-irritated, scratching the face; 2-hair in an upright state; 3-reduced activity and yellow viscosity with faeces; 4-shortness of breath, erect hair, and white lips; and 5-death.²⁵

2.5. Analysis of specific IgG, IgG1, IgG2a, and IgA against β -CG by enzyme-linked immunosorbent assay (ELISA)

Specific immunoglobulin G (IgG) antibodies against β -CG, including immunoglobulin G1 (IgG1), immunoglobulin G2a (IgG2a), immunoglobulin A (IgA) and the related ELISA kit were purchased from Shanghai Enzyme Biotechnology Co., Ltd. The levels of specific IgG, IgG1, and IgG2a in the serum and IgA in the small intestine were determined using the prepared ELISA kits according to the manufacturer's instructions.

2.6. Analysis of serum IgE, HIS, MCP, MCT, and cytokines in spleen cells by ELISA

Determination of IgE, histamine (HIS), monocyte chemoattractant protein (MCP), and mast cell tryptase (MCT) levels in serum were performed with ELISA kits (Nanjing Jiancheng

Bioengineering Institute, Nanjing, China)²⁶ according to the manufacturer's instructions.

Th2-related cytokines of spleen cells (interleukin 17A (IL-17A), transforming growth factor- β (TGF- β), interleukin 4 (IL-4), interleukin 10 (IL-10), interleukin 13 (IL-13), and interleukin 5 (IL-5)), and Th1-related cytokines of spleen cells (IFN- γ and interleukin 12 (IL-12)) were determined using ELISA kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), according to the manufacturer's instructions.

2.7. Analysis of spleen cell proliferation

The proliferation of spleen cells was determined using a cck-8 kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

2.8. Flow cytometry analysis

The spleen was fully ground on a 200-mesh sieve, mono-cells were collected by centrifugation at 1000 rpm for 5 min at 4 °C. The pelleted cells were resuspended in 5 mL of red blood cell lysate and centrifuged at 1000 rpm for 5 min. The concentration of spleen cells was adjusted to 10^6 mL^{-1} after counting. Five microlitres of antibody (FITC-labelled CD8a, PE-labelled interferon- γ (IFN- γ), PE-labelled interleukin4 (IL-4), PE-labelled CD3, Per CP-Cy5.5-labelled CD19, PE-labelled CD4) (eBiosciences, San Diego, USA) was added to each 500 μL of cell suspension, incubated for 30 min at 37 °C, and then centrifuged at 1000 rpm for 5 min. The cells were resuspended in an appropriate amount of phosphate buffered saline (PBS) (protected from light) for flow cytometry analysis. Data were obtained with a BD FACSARIATM and analysed using the BD FACSDiva Software.

2.9. IHC analysis

The paraformaldehyde-fixed duodenum was applied to a Leica semi-automatic rotary slicer for the preparation of paraffin sections with 4 μm thickness. The tissue sections were exposed to 45 °C warm water using a stretcher. After the sections were spread, the tissue sections were fixed on a glass slide for 4 h at 60 °C. The specific procedure, including serum blocking, adding primary antibody, adding sensitizer, and adding secondary antibody, was carried out according to the instructions of an immunohistochemical EliVisionTM kit (rat/rabbit).

2.10. Transcriptome sequencing and data analysis

The transcriptome sequencing of the spleen was performed by Beijing Novogene Bioinformatics Technology Co., Ltd. A total amount of 3 μg of RNA per sample was used as the input material for the RNA sample preparations. Sequencing libraries were generated using the NEBNext[®] UltraTM RNA Library Prep Kit for Illumina[®] (NEB, USA) following the manufacturer's recommendations and the index codes were added to attribute the sequences to each sample. All downstream analyses were based on high-quality clean data. The index of the reference genome was built using Hisat2 v2.0.5, and paired-end clean reads were aligned to the reference genome using Hisat2 v2.0.5. The FPKM of each gene was calculated

based on the length of the gene and read count mapped to this gene. The differential expression analysis of the two groups (three biological replicates per condition) was performed using the DESeq2 R package. Gene ontology (GO) enrichment analysis of the differentially expressed genes (DEGs) was performing using the cluster Profiler R package. We used the cluster Profiler R package to also test the statistical enrichment of DEGs in the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.

2.11. qPCR analysis

RNA of spleen was extracted using a TOYOBO One-step qPCR Kit (Bio-Rad, Hercules, USA). One microgram of RNA was reverse transcribed using a LunaScriptTM RT-SuperMix Kit. The primers used in the current study are listed in Table 1. The relative quantities of each transcript were normalized to β -actin in the same cDNA and data were analysed according to the $2^{-\Delta\Delta\text{Ct}}$ method. The cycling conditions were 95 °C for 5 min (60 °C for 10 s, 72 °C for 15 s, and 95 °C for 15 s) $\times$ 45, and 60 °C for 1 min, and 95 °C for 15 s.

2.12. Statistical analysis

Data are expressed as the mean $\pm$ standard deviation. All data were analysed using the SPSS 17.0 software (SPSS Inc., Illinois, Chicago, USA). Where appropriate, a one-way ANOVA nonparametric test was performed to determine statistical significance. *P* values <0.05 were considered statistically significant.

3. Results

3.1. The establishment of the β -CG induced allergy mouse model

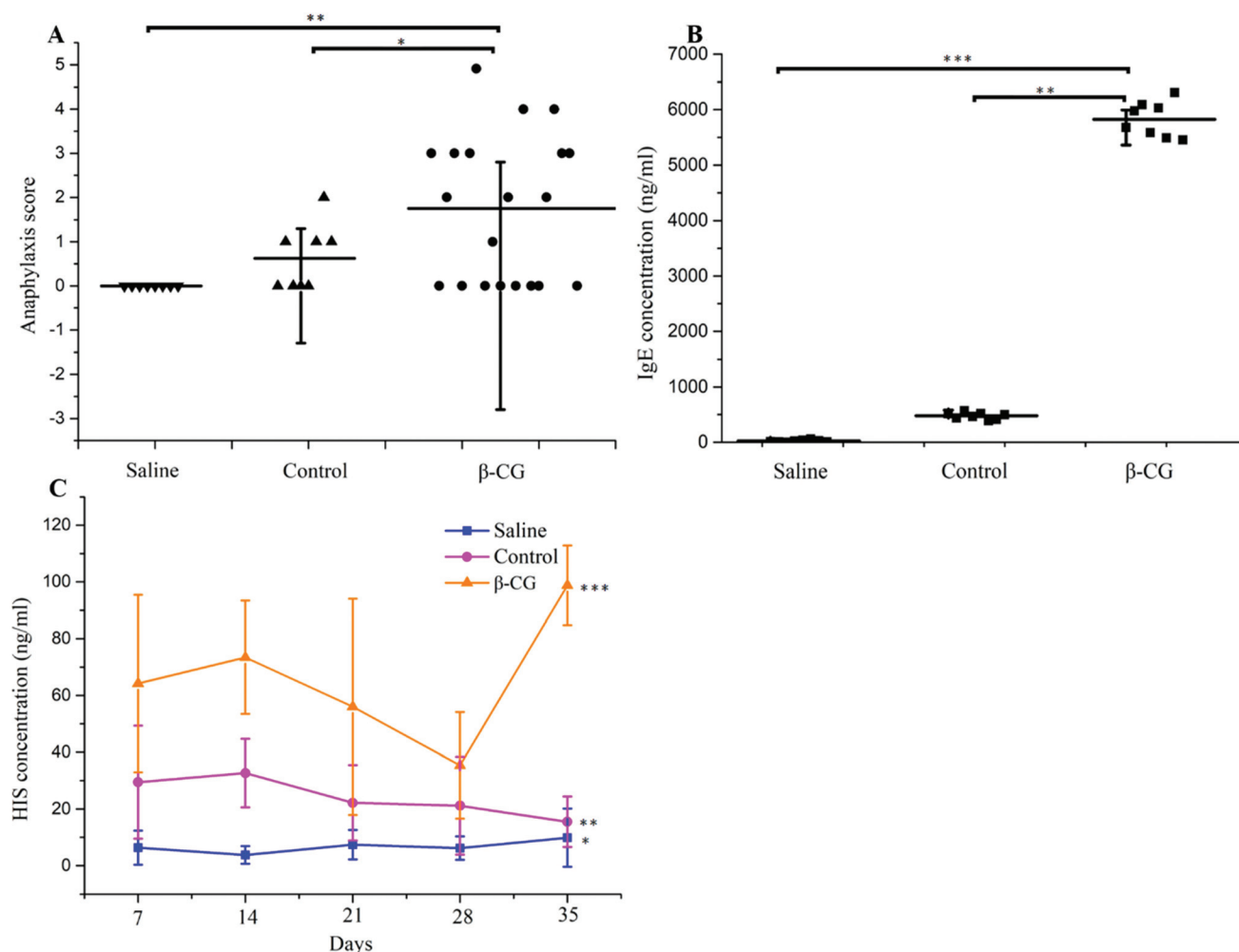
In the current study, the β -CG-induced allergy model was established for the first time. The allergy symptoms of the mice were scored by observing their appearance. No abnormalities were observed in the saline group and the control group mice. In contrast, the mice in the β -CG group showed obvious allergic clinical symptoms, including hair erection, shortness of breath, diarrhoea and decreased activity (Fig. 1A). Because IgE and HIS are important clinical indicators for the diagnosis of allergies, the levels of IgE and HIS in serum were determined. Compared with the saline group and control group, the levels of both IgE and HIS were increased significantly in the β -CG group (Fig. 1B and C). In conclusion, based on the allergy symptoms and the levels of IgE and HIS, the β -CG-induced allergy mouse model was successfully established.

3.2. LGG alleviates the allergic response induced by β -CG

After successfully establishing a β -CG induced allergy model, the intervention effect of LGG on β -CG-induced allergies was evaluated. The allergic symptoms of LGG treatment were significantly reduced, and the relief effect of LGG treatment was better than that of NR treatment (Fig. 2A). The HIS content of the LGG-1 group showed a more significant decreasing trend than that of the NR-1 group (Fig. 2B). The levels of MCT and

Table 1 Primers used in qPCR reactions

Gene	Forward primer(5' → 3')	Reverse primer(5' → 3')
<i>IL-4</i>	ACAGGAGAAGGGACGCCAT	GAAGCCCTACAGACGAGCTCA
<i>IL-13</i>	AGACCAGACTCCCTGTGCA	TGG-GTCCTGTAGATGGCATTG
<i>IFN-γ</i>	TGGCATAGATGTGGAAGAAAAGAG	TGCAGGATTTTCATGTCACCA
<i>TGF-β</i>	ACCGCAACAACGCCATCTAT	GCACTGCTTCCCGAATGTCT
<i>β-Actin</i>	GTGGGGCGCCCCAGGCACCA	CTCCTTAATGTACGCACGATTTC

**Fig. 1** Establishment of β -CG-induced allergy mouse model. (A) The score of anaphylaxis symptoms. (B) Levels of IgE. (C) Levels of HIS (* p < 0.05; ** p < 0.01; *** p < 0.001).

MCP in the β -CG group were significantly higher than those in the saline group and the control group (Fig. 2C) but significantly reduced after LGG intervention (Fig. 2D). As expected, the contents of IgG2a, IgG, and IgG1 increased in allergic mice and showed a downward trend after LGG intervention (Fig. 2E–G). The above results indicated the beneficial effects of LGG intervention on reducing the serum levels of inflammatory factors and immunoglobulins. Because IgA is involved in the intestinal barrier function and plays an important role in maintaining immune homeostasis, specific IgA levels in the small intestine contents were evaluated. The levels of specific

IgA in the LGG-1 group and NR-1 group were significantly lower than those in the β -CG group, especially the LGG-1 group (Fig. 2H), which was consistent with the trends of IgE and HIS.

CD4⁺ T cells are important immune cells in the intestinal immune system. We used immunohistochemistry to detect the infiltration of CD4⁺ T cells in the duodenum of mice (Fig. 3). We observed that the infiltration of CD4⁺ T cells was increased, the length of intestinal villi was decreased, and the intestinal villi appeared to be broken, were in disarray, and even broke off into the intestinal cavity. A large number of villi

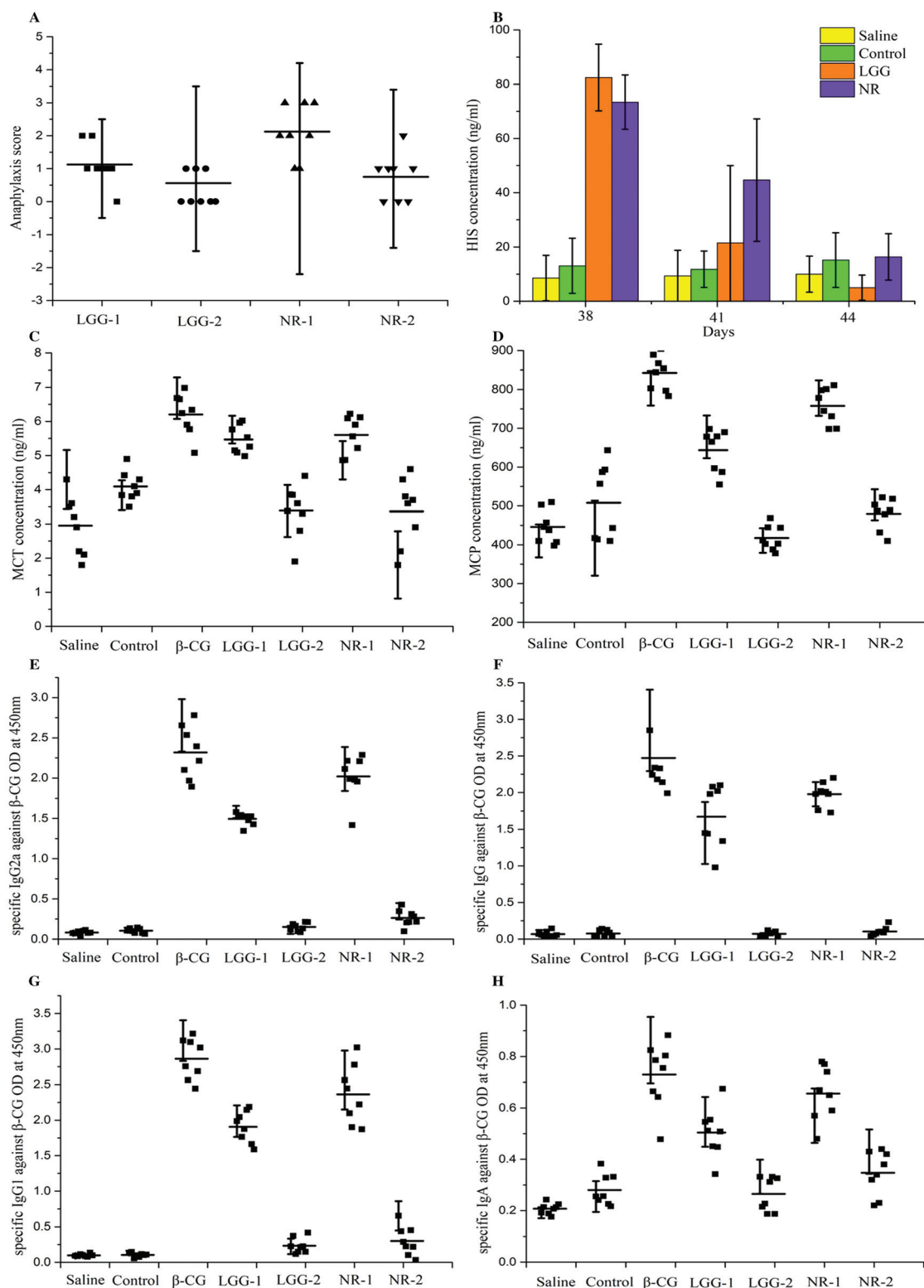


Fig. 2 Effect of LGG intervention on inflammatory factors in the serum. (A) The score of anaphylaxis symptoms. (B) Levels of HIS. (C) Levels of MCT. (D) Levels of MCP. (E) Levels of IgG2a. (F) Levels of IgG. (G) Levels of IgG1. (H) Levels of IgA (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

fragments were observed in the large intestine. The muscle layer of the mucosa was thickened and the crypt was severely twisted in the β -CG group. After LGG intervention, the infiltra-

tion of CD4⁺ T cells decreased and intestinal villi recovered to varying degrees, especially the LGG recovery effect, which was better than that in the NR group. Almost all the intestinal villi

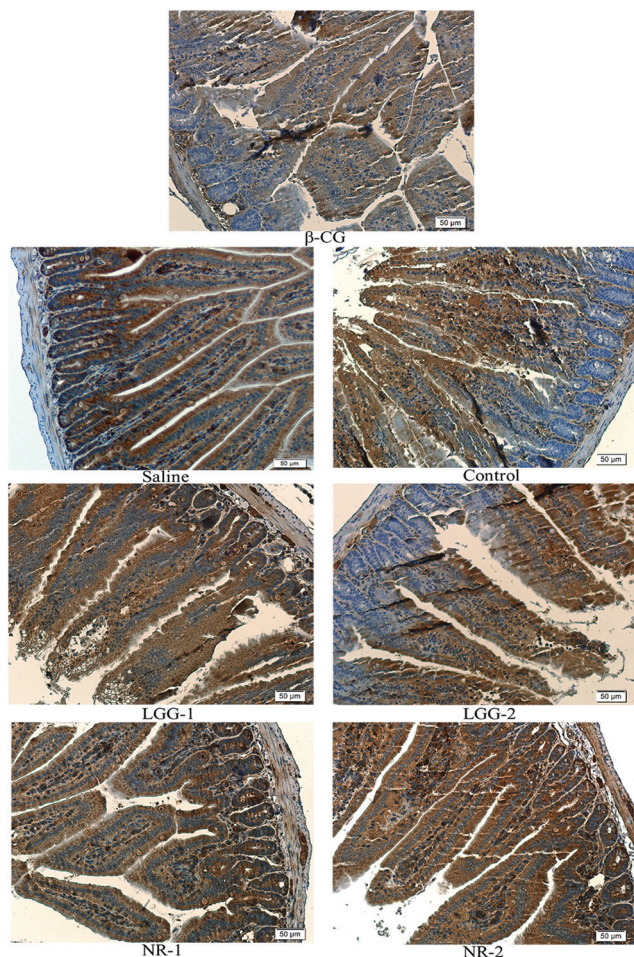


Fig. 3 Immunohistochemistry of mouse duodenal CD4⁺ T cell (magnification $\times 40$).

in the LGG-2 group recovered. The intestinal villi and epithelial cells were arranged neatly. The mucosal muscle layer was similar to that of the saline group and distributed evenly. The results indicated that LGG treatment effectively decreased the infiltration of CD4⁺ T cells and protected the structural integrity of the intestinal mucosa. In summary, LGG had a significant recovery effect on intestinal immune homeostasis.

3.3. The effect of LGG intervention on T-cell differentiation

To further investigate the underlying mechanism of the beneficial effect of LGG, the effect of LGG on the proliferation ability of spleen cells and T-cell differentiation was studied. The proliferation of spleen cells *in vitro* is usually used to describe the body's immune response. The results showed that after β -CG stimulation, the proliferation ability of spleen cells was significantly higher than that in that saline group and control group, while it returned to normal after LGG intervention (Fig. 4). The results showed that LGG intervention weakened the body's immune stress state and restored immune homeostasis.

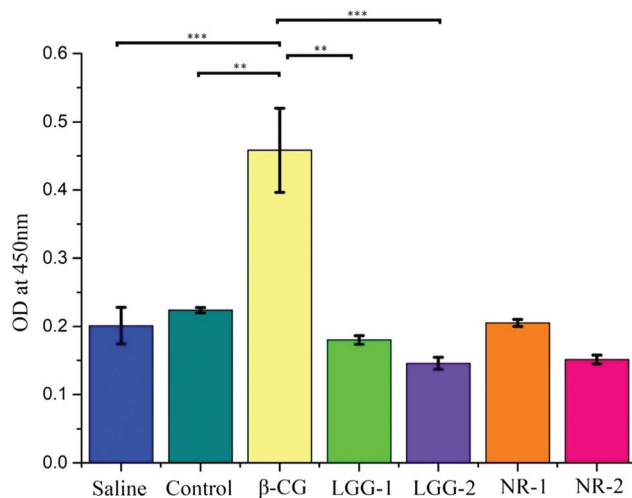


Fig. 4 Effect of LGG intervention on spleen cell proliferation *in vitro* (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

In the spleen of normal mice, the ratio of the number of T cells to B cells is approximately 1. With CD3 cells and CD19 cells as representatives, the differentiation of T cells and B cells in spleen cells was studied. The results showed that the β -CG challenged mice had a higher proportion of CD19, resulting in a lower ratio of CD3/CD19, and the ratio of CD3/CD19 increased close to 1 after LGG intervention (Fig. 5A), which proved that the ratio of T/B cells was balanced after LGG intervention. In β -CG-stimulated mice, the ratio of CD4/CD8 α cells increased, and after LGG intervention, the ratio of CD4/CD8 α cells decreased (Fig. 5B). On the basis of studying the differentiation of T cells and B cells, the differentiation of Th cells was assessed. Under normal circumstances, the proportion of Th1 cells is large. When stimulated by allergens, the proportion of Th1 cells decreases and the proportion of Th2 cells increases. IFN- γ and IL-4 represent Th1 cells and Th2 cells, respectively. As shown in Fig. 5C and D, IFN- γ increased and IL-4 decreased after LGG intervention. The results suggested that LGG inhibited Th2 cell differentiation better than NR.

Th cytokines play an important role in the immune response; hence, cytokines isolated from spleen tissue were further analysed. Th2 cytokines (IL-17A, TGF- β , IL-4, IL-10, IL-13, and IL-5) increased in allergic mice (Fig. 6A–F), and decreased levels of Th2 cytokines were observed after LGG intervention. In addition, Th1 cytokine (IFN- γ , IL-12) levels were increased after LGG intervention (Fig. 6G and H). In summary, the comprehensive evaluation of spleen cell proliferation ability, T/B cells, Th1/Th2 cells and Th1/Th2 cytokine levels showed that LGG could reduce the proliferation ability of B cells and Th2 cells and regulate T-cell differentiation, thus alleviating β -CG-induced allergy.

3.4. LGG alleviates β -CG-induced allergies by regulating the TCR signaling pathway

To further reveal the molecular mechanisms of the alleviating effect of LGG regarding β -CG-induced allergy, the gene

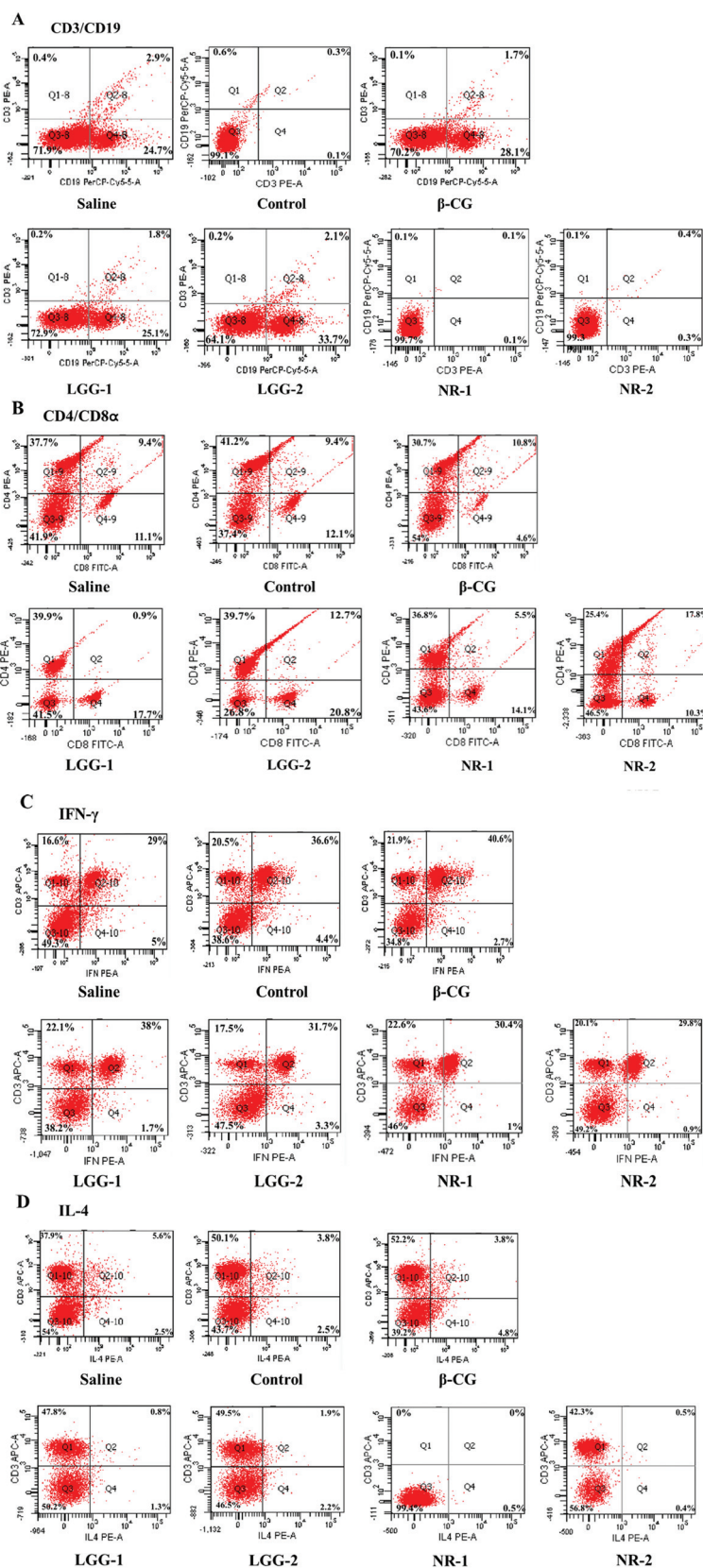


Fig. 5 Effect of LGG intervention on the proliferation ability *in vitro*. (A) Proportion of CD3/CD19. (B) Proportion of CD4/CD8α. (C) Proportion of IFN-γ. (D) Proportion of IL-4.

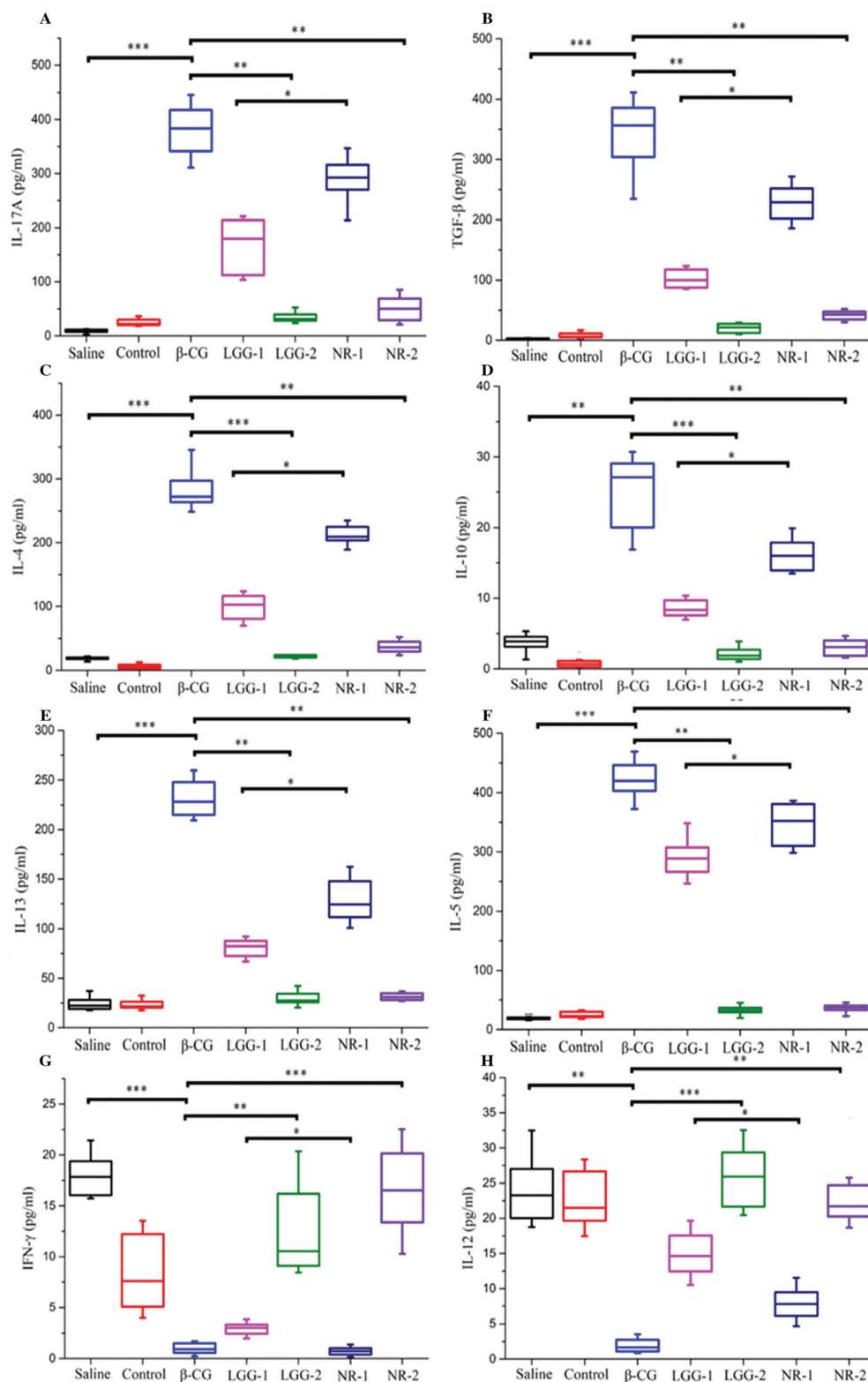


Fig. 6 Effect of LGG intervention on inflammatory cytokine content in spleen cells. (A) Levels of IL-17A cytokines. (B) Levels of TGF- β cytokines. (C) Levels of IL-4 cytokines. (D) Levels of IL-10 cytokines. (E) Levels of IL-13 cytokines. (F) Levels of IL-5 cytokines. (G) Levels of IFN- γ cytokines. (H) Levels of IL-12 cytokines (* p < 0.05; ** p < 0.01; *** p < 0.001).

expression of spleen cells was evaluated by transcriptome sequencing. GC content distribution showed the relative equal contents of GC and AT in each position, which serve as the evidence to demonstrate the consistency throughout the sequencing process (Fig. S1†). The results of the Pearson correlation between the samples and PCA demonstrated the significant differences between groups with credible duplication (Fig. S2 and S3†). We performed differential expression analysis for different treatment comparisons, including comparing the LGG-1 group and NR-1 group, LGG-2 group and β -CG group. Hierarchical clustering of the samples based on DEGs clearly separated the LGG-1 group and NR-1 group, LGG-2 group and β -CG group. When the same gene in the LGG-2 group was highly expressed, the β -CG group was expressed at low levels, and when the same gene in the LGG-2 group was expressed at low levels, the β -CG group was highly expressed (Fig. 7). The heatmap results indicated that genes with similar expression patterns converged together and may have a common function or participate in a common metabolic pathway or a common signaling pathway.

GO analysis is used to describe the properties of genes and their products in any organism. A total of 16 147 unigenes were assigned predicted GO terms, which were summarized into three categories: biological processes (BP), cellular components (CC) and molecular functions (MF) ($p_{adj} < 0.05$). The results showed that compared with the NR-1 group, in the LGG-1 group, a large number of gene products were identified in the BP, such as T cell activation (9.48%) and B cell activation (7.4%); immunoglobulin complexes (9.6%) and MHCII protein complexes (1.35%) were identified in CC; and antigen binding (5.9%) was identified in MF (Fig. 8A). Compared to the β -CG group, in the LGG-2 group, a large number of gene products were identified in the BP, such as T cell differentiation (2.4%) and T cell activation (4%); MHCII protein complexes (0.19%) were identified in CC; and GTPase regulator activity (3.9%) was identified in MF (Fig. 8B).

For KEGG enrichment, the 20 most significant signaling pathways were selected ($p_{adj} < 0.05$). Compared to the NR-1 group, the LGG-1 group DEGs were involved in immune-related signaling pathways, including the Th1 and Th2 cell differentiation pathways, the Th17 cell differentiation pathway and the intestinal immune network for IgA production, which were perceived as the most enriched pathways (Fig. 9A). The results showed that the DEGs were mainly involved in the intestinal immune network for IgA production. Compared to the β -CG group, the LGG-2 group had significantly enriched the TCR signaling pathway (Fig. 9B). The results indicated that the TCR signaling pathway played a crucial role in T cell differentiation during LGG intervention in β -CG-induced allergies.

Several immune-related genes that were upregulated or downregulated in the TCR signaling pathway were selected according to the gene expression threshold FPKM > 1 , $p_{adj} < 0.05$ and $|\log_2(\text{Foldchange})| > 1$. Compared with the NR-1 group, 202 differentially expressed mRNAs were upregulated, and 344 differentially expressed mRNAs were downregu-

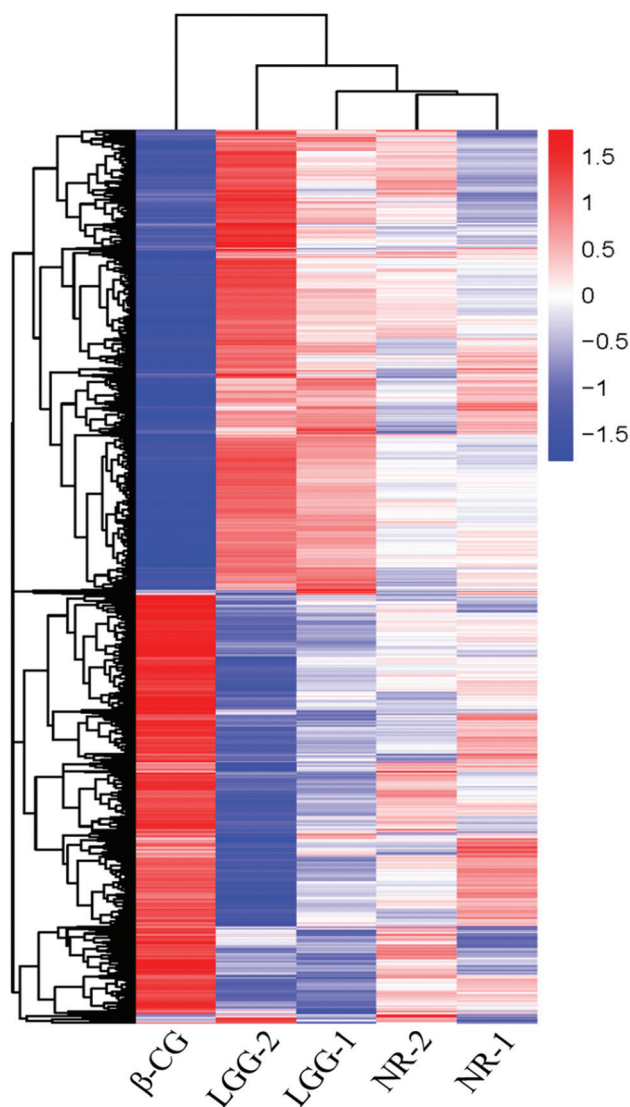


Fig. 7 The cluster analysis of differential genes in spleen was shown by a heatmap.

lated in the LGG-1 group. *Il4ra* was significantly downregulated and *Il12ra* was significantly upregulated (Fig. 10A). Compared to the β -CG group, 2305 differentially expressed mRNAs were upregulated and 1801 differentially expressed mRNAs were downregulated in the LGG-2 group, including *Tgfb2*, *Il13ra2*, and *Il4ra*, which were significantly downregulated, and *Ifnar2* was significantly upregulated (Fig. 10B). The results revealed that the levels of *Ifnar2* and *Il12ra* gene expressions were upregulated after LGG intervention.

qPCR results further verified the genes in the TCR signaling pathway in which the level of IFN- γ mRNA was high and IL-4 mRNA, IL-13 mRNA and TGF- β mRNA were low after LGG intervention (Fig. 11A–D). The results showed that LGG played a critical role in regulating the level of immune-related mRNA expression.

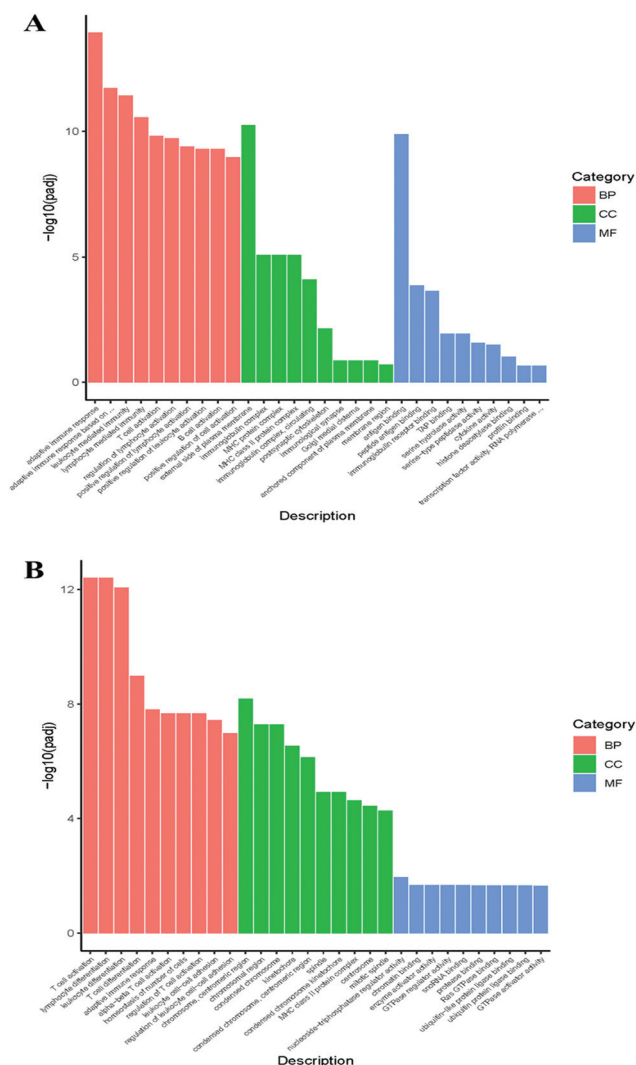


Fig. 8 Effect of LGG intervention on differential genes function in the spleen. (A) Analysis of GO function enrichment between the LGG-1 group and NR-1 group. (B) Analysis of GO function enrichment between the LGG-2 group and β -CG group.

4. Discussion

The updated results related to the intervention effect of LGG on the alleviated allergic response have been published including the explanation from varied aspects. However, no related report about the LGG intervention on β -CG-induced allergy has been revealed; especially, the potential mechanism remains to be further explored *in vivo*. The current study demonstrated the LGG intervention related alleviation of allergic symptoms, inflammatory response, duodenal mucosa damage, and CD4⁺ T cell infiltration, as well as the underlined mechanism through the TCR signaling pathway.

The well establishment of the β -CG-induced allergy model was verified by different approaches, including the allergy symptom score and the levels of IgE and HIS. Upon the appearance of the clinical symptoms of allergies, IgG, IgG1

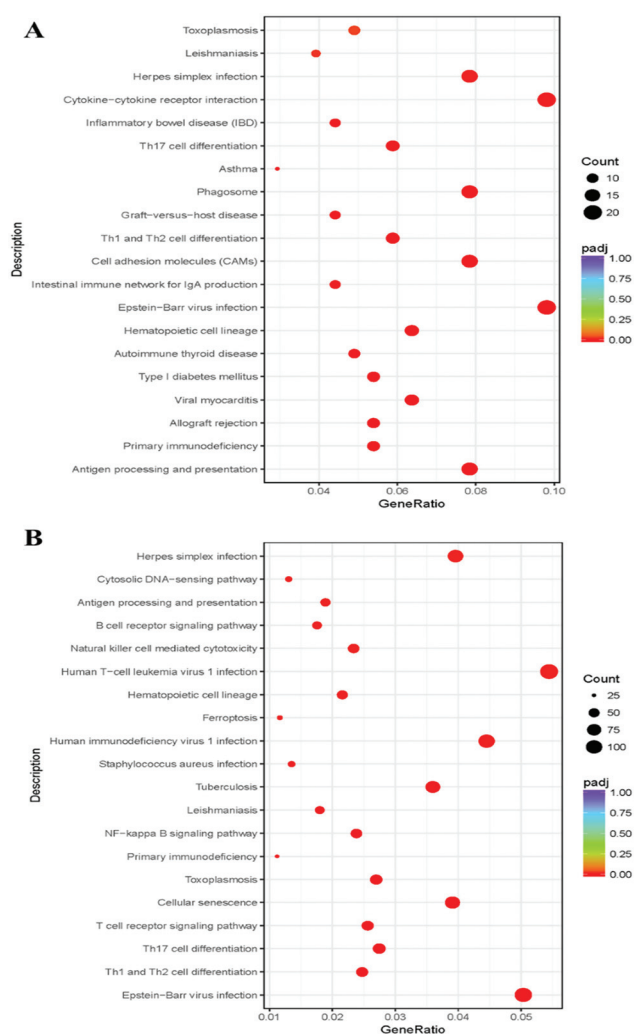


Fig. 9 Effect of LGG intervention on differential gene pathways in the spleen. (A) Analysis of KEGG pathway enrichment between the LGG-1 group and NR-1 group. (B) Analysis of KEGG pathway enrichment between the LGG-2 group and β -CG group.

and IgG2a are usually secreted by B cells, and HIS, MCT, and MCP are released.²⁷ Therefore, the intervention effect of LGG on β -CG-induced allergies was further assessed by detecting the contents of the typical indicators for allergies.

The above-mentioned experimental results demonstrated the intervention effect of LGG on the inflammatory factors in the serum of allergic mice. Further research has been performed to reveal the effect on cytokines in spleen cells. CD4 cells are important immune cells expressed on helper T (Th) cells. CD4 cells participate in the TCR recognition of antigens by the Th cells and bind to the nonpeptide region of the major histocompatibility complex (MHC) class II (MHCII) molecule.²⁸ CD8 α recognizes the specific antigen presented by its own MHCII molecule through TCR and enhances the interaction between T cells and antigen presenting cells (APCs) or target cells. The results demonstrated that LGG intervention weakens antigen recognition and enhances the interaction between T cells and target cells.

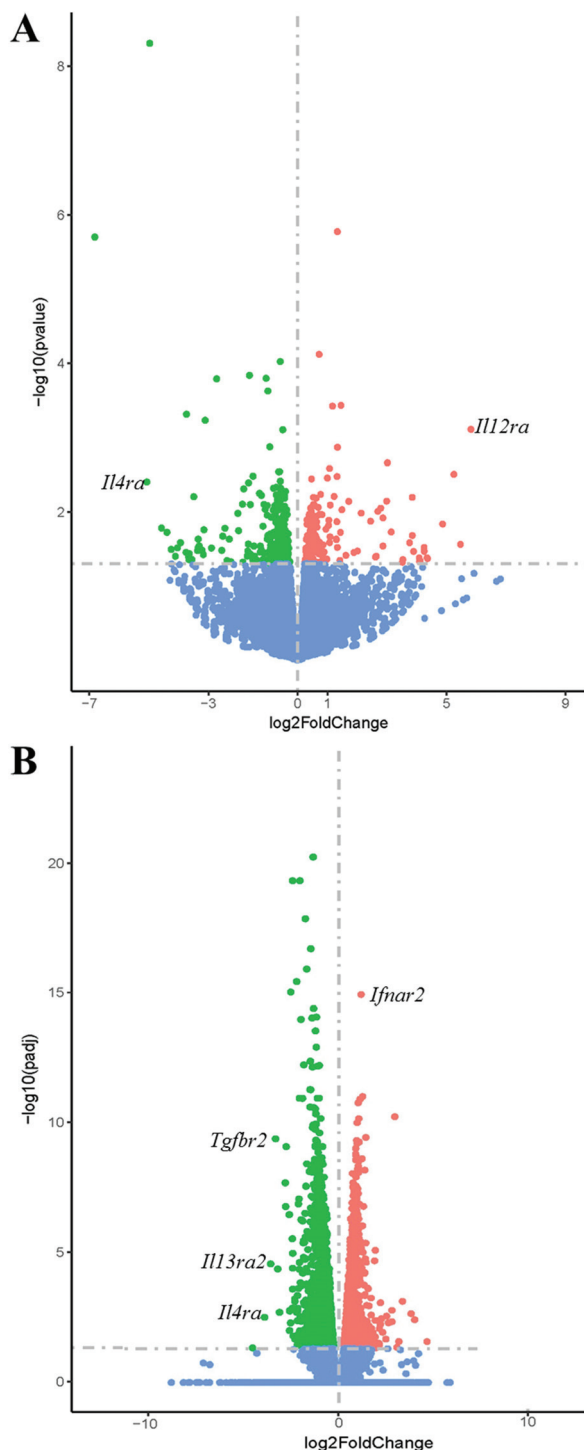


Fig. 10 Effect of LGG intervention on differential gene distribution in the spleen. (A) Volcano map of differential gene distribution between the LGG-1 group and NR-1 group. (B) Volcano map of differential gene distribution between the LGG-2 group and β-CG group.

IL-4 is a highly potent and multipotent cytokine that acts on T cells and promotes their own proliferation. IL-10 is a cytokine produced by dendritic cells that can promote the production of T cells and other immune cells. TGF-β can

resist or improve the role of other cytokines and participate in immune tolerance. Th2 cytokines inhibited the differentiation of Th1 cytokines.²⁹ IFN-γ produced by Th1 cells promotes the secretion of Th1 cytokines and inhibits the secretion of Th2 cytokines. Therefore, IFN-γ was significantly reduced in allergic mice, indicating a shift from the Th1 to Th2 response, resulting in a Th1/Th2 imbalance. Upon LGG intervention, the ratio of Th1/Th2 cytokines was kept in balance, which was consistent with the study of allergic airway inflammation in mice by LGG intervention during pregnancy.³⁰

From the above GO enrichment results, LGG was further identified to be involved in the regulation of allergic inflammation and affected the differentiation of regulatory T cells. MHCII is mainly expressed in B cells, and the expression of MHCII can be regarded as a sign of antigen presentation ability. The function of MHCII is mainly to present antigens to CD4⁺ T cells in the immune response, which was consistent with the conclusion that the above LGG intervention weakened the proliferation of T cells. On the external side of the plasma membrane, the activity of RasGTPase is closely related to the guanine nucleotide exchange factor (GEF) and GTPase activating protein (GAP). Its functions are to promote RasGTPase release protein and activate RasGTPase. RasGTPase plays an important role in T cell differentiation, which is consistent with the conclusion of regulating T cell differentiation after LGG intervention.

The differentiation of B cells was evaluated as well. IgA plays a very important role in protecting the body from microbial invasion on the mucosal surface. After antigen processing and presentation, IgA type B cells are formed against the antigen. In this process, the differentiation and proliferation of B cells depend on T cells, including the Th2 cytokines TGF-β, IL-4, IL-10, etc. In particular, IL-4 is very important for the synthesis of IgA, which is consistent with the results of the change in IgA content after LGG intervention.

In this study, the intervention effect of LGG on β-CG-induced allergy has been determined after performing the KEGG pathway enrichment analysis. The TCR signaling pathway is closely related to T cell homeostasis.³¹ TCR antigen recognition and subsequent TCR-mediated signaling pathway activation are essential for initiating Th cell differentiation.³² Transcriptome analysis revealed the role of LGG in immune-related genes. The intervention of LGG inhibited the expression of the *Il4ra* and *Il13ra2* genes in the spleens of allergic mice. *IL-4ra* is a subunit shared by IL-4r and IL-13r, which mainly transduces the signals of the IL-4 and IL-13 pathways.³³ Therefore, when *IL-4ra* was decreased, IL-4 and IL-13 were also decreased. *Ifnar2* is critical for signal transduction through the receptor.³⁴ As a result, the genes associated with TCR signaling pathways in β-CG-induced allergies, including *Il4ra*, *Il13ra2* and *Tgfb2*, were upregulated and *Ifnar2* was downregulated. After LGG intervention, *Il4ra*, *Il13ra2* and *Tgfb2* were downregulated and *Ifnar2* was upregulated. In addition, the mRNA level of the related genes in the TCR signaling pathway was verified by qPCR (Fig. 11A–D).

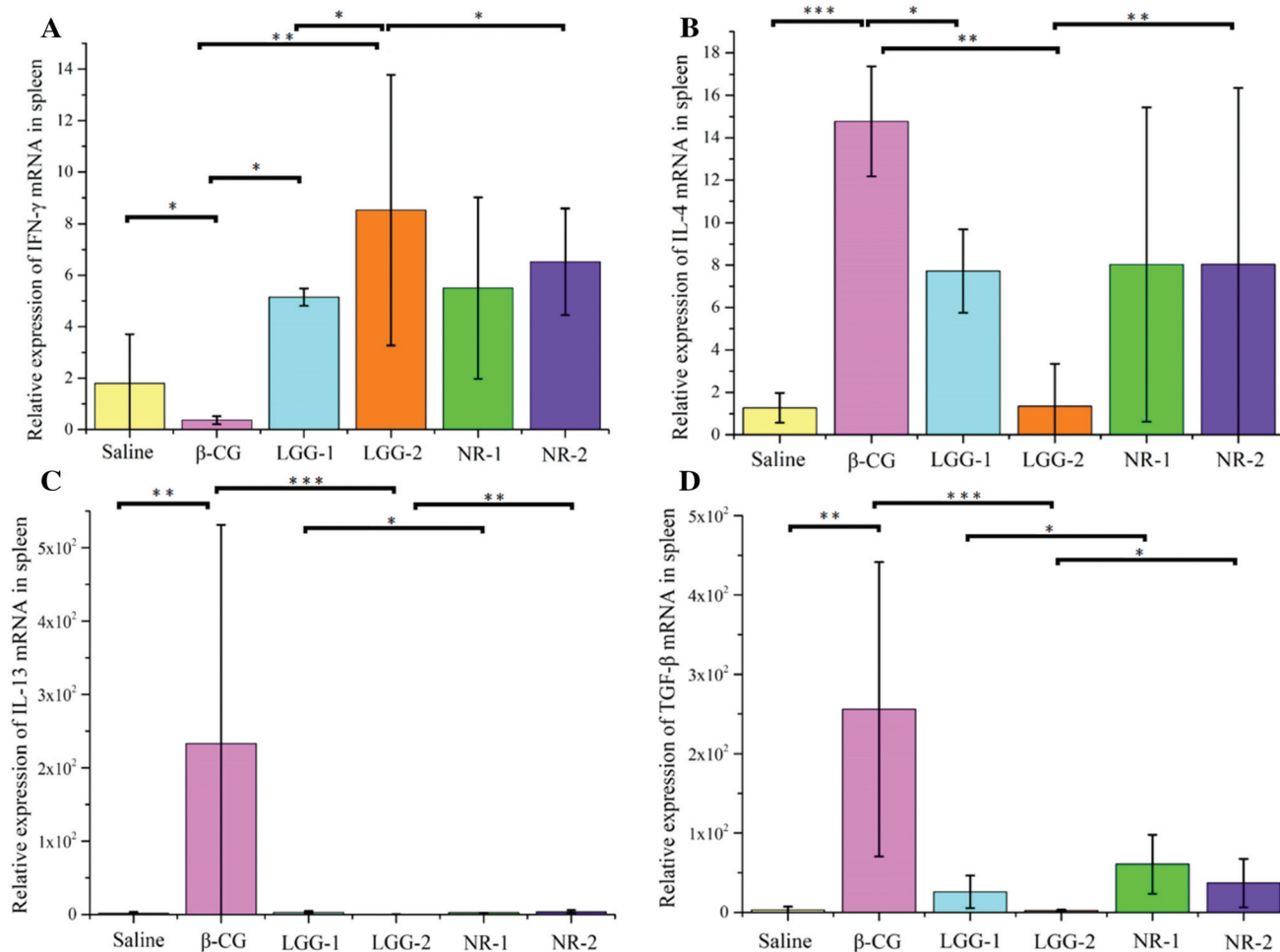


Fig. 11 Effects of LGG intervention on relative mRNA expression in the spleen. (A) Relative expression of IFN- γ mRNA. (B) Relative expression of IL-4 mRNA. (C) Relative expression of IL-13 mRNA. (D) Relative expression of TGF- β mRNA (* p < 0.05; ** p < 0.01; *** p < 0.001).

Furthermore, the variation of related cytokines, including IL-4, IL-13, TGF- β , and IFN- γ , were confirmed by ELISA (Fig. 6A–H). Hence, LGG played a vital role in initiating T cell differentiation through the TCR signaling pathway.

5. Conclusion

Based on the establishment of a β -CG allergy mouse model, the therapeutic effect of LGG on β -CG-induced allergies was identified. LGG could effectively alleviate the allergic response, restore the levels of HIS, IgE, MCP, MCT, specific IgG, specific IgG1, specific IgG2a and other inflammatory factors, and restore CD4⁺ T cell infiltration and the status of intestinal villi. LGG intervention could significantly decrease the proliferation of spleen cells and regulate T-cell differentiation to maintain the balance of Th1/Th2 and Th17/Treg by regulating the TCR signaling pathway. The current study enhances the application of LGG and ultimately provides the basis for dietary intervention for food allergies.

Abbreviations

LGG	<i>Lactobacillus rhamnosus</i> GG
β -CG	β -Conglycinin
Tregs	Regulatory T cells
IgE	Immunoglobulin E
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgG1	Immunoglobulin G1
IgG2a	Immunoglobulin G2a
IFN- γ	Interferon- γ
TGF- β	Transforming growth factor- β
IL-4	Interleukin 4
IL-5	Interleukin 5
IL-17A	Interleukin 17A
IL-10	Interleukin 10
IL-12	Interleukin 12
IL-13	Interleukin 13
CT	Cholera toxin
HIS	Histamine
MCP	Monocyte chemoattractant protein

MCT	Mast cell tryptase
FACS	Fluorescence activated cell sorting
qPCR	Real-time quantitative polymerase chain reaction
IHC	Immunohistochemical
TCR	T cells receptor
Th	Helper T
MHC	Major histocompatibility complex
MHCII	MHC class II
GEF	Guanine nucleotide exchange factor
GAP	GTPase activating protein
ELISA	Enzyme-linked immunosorbent assay
KEGG	Kyoto encyclopedia of genes and genomes
BP	Biological process
CC	Cellular component
MF	Molecular function

Conflicts of interest

There are no conflicts of interest to declare.

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Supplementary Materialfor

Lactobacillus rhamnosus GG alleviates β -conglycinin induced allergy by regulating the T cell receptor signaling pathway

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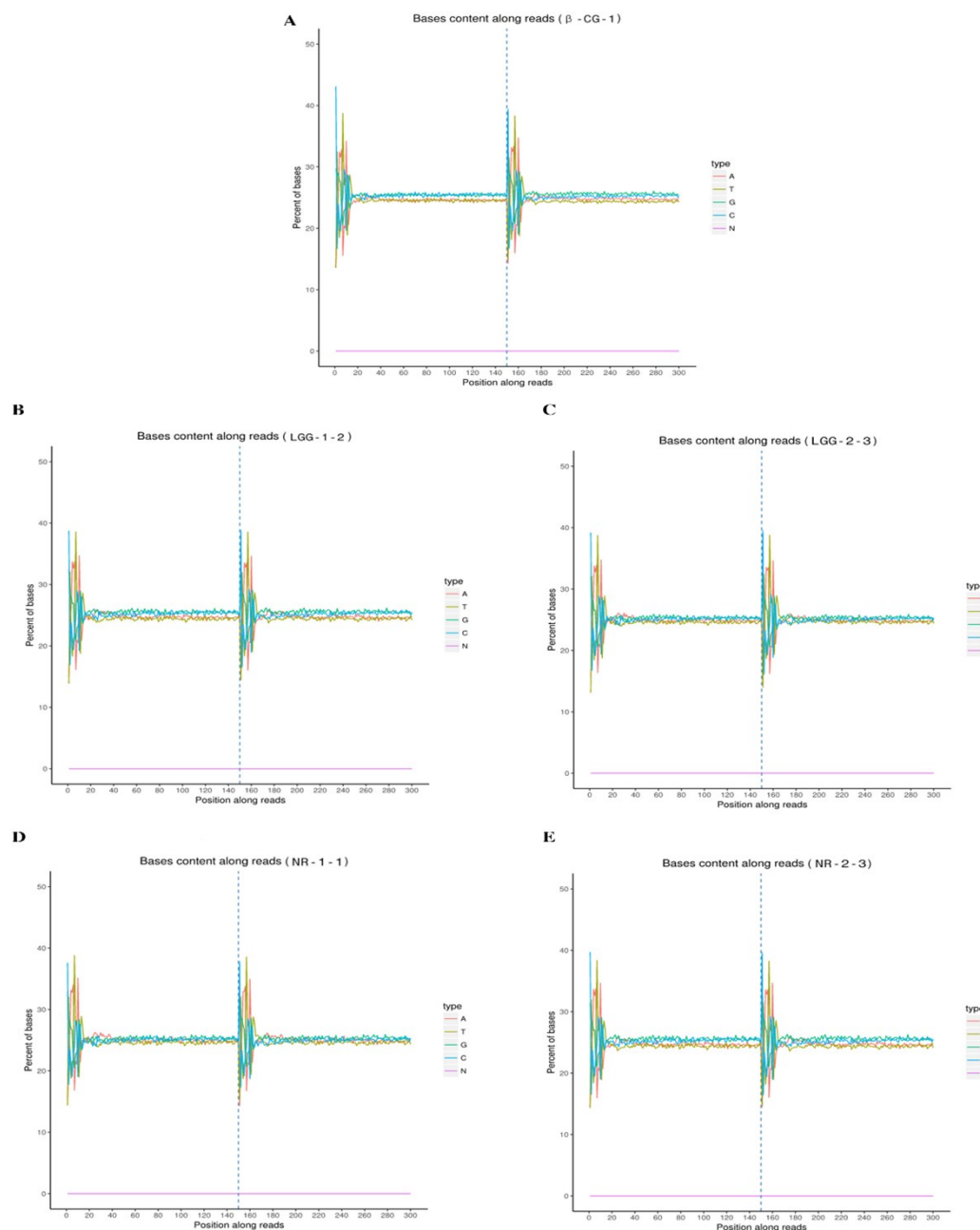


Figure S1 GC content distribution diagram. (A) Distribution of GC content in β -CG group. (B) Distribution of GC content in LGG-1 group. (C) Distribution of GC content in LGG-2 group. (D) Distribution of GC content in NR-1 group. (E) Distribution of GC content in NR-2 group.

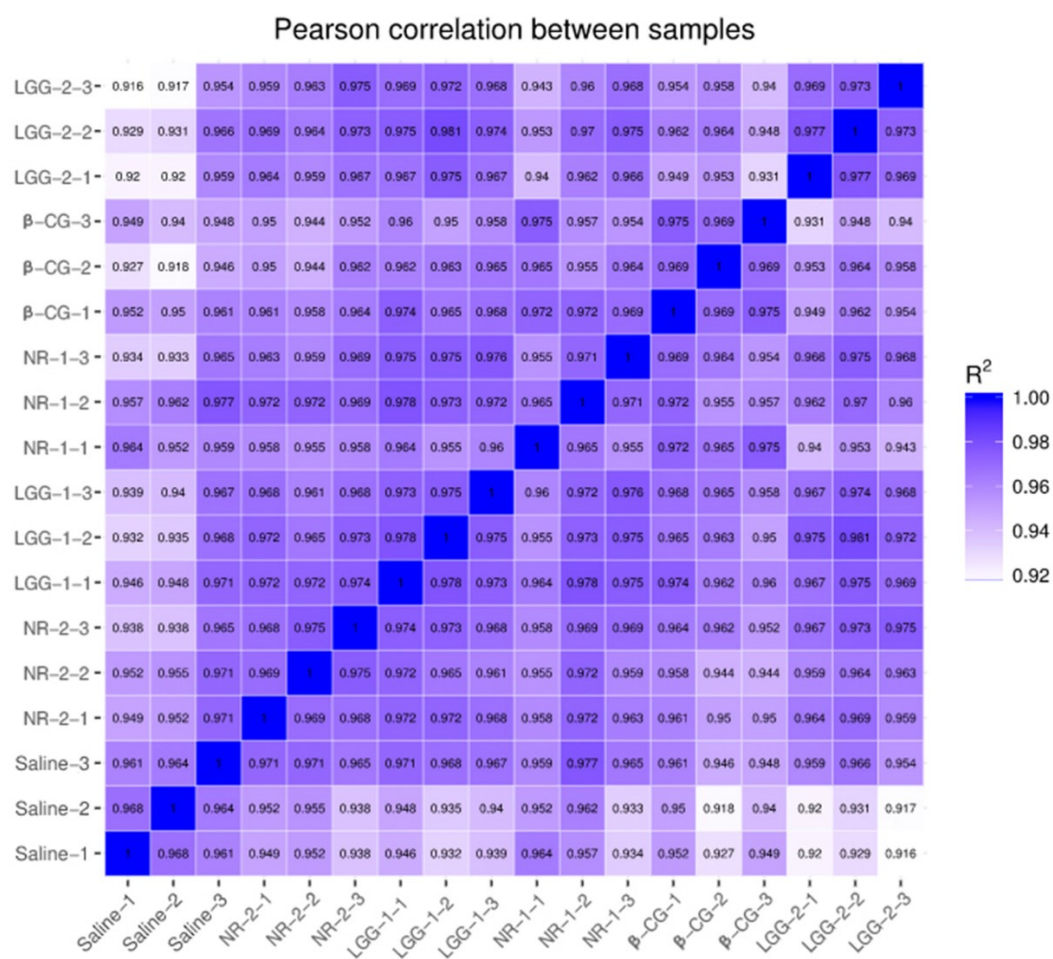


Figure S2 Correlation of gene expression levels between samples in saline group, β-CG group, LGG-1 group, LGG-2 group, NR-1 group and NR-2 group based on RNA of spleen.

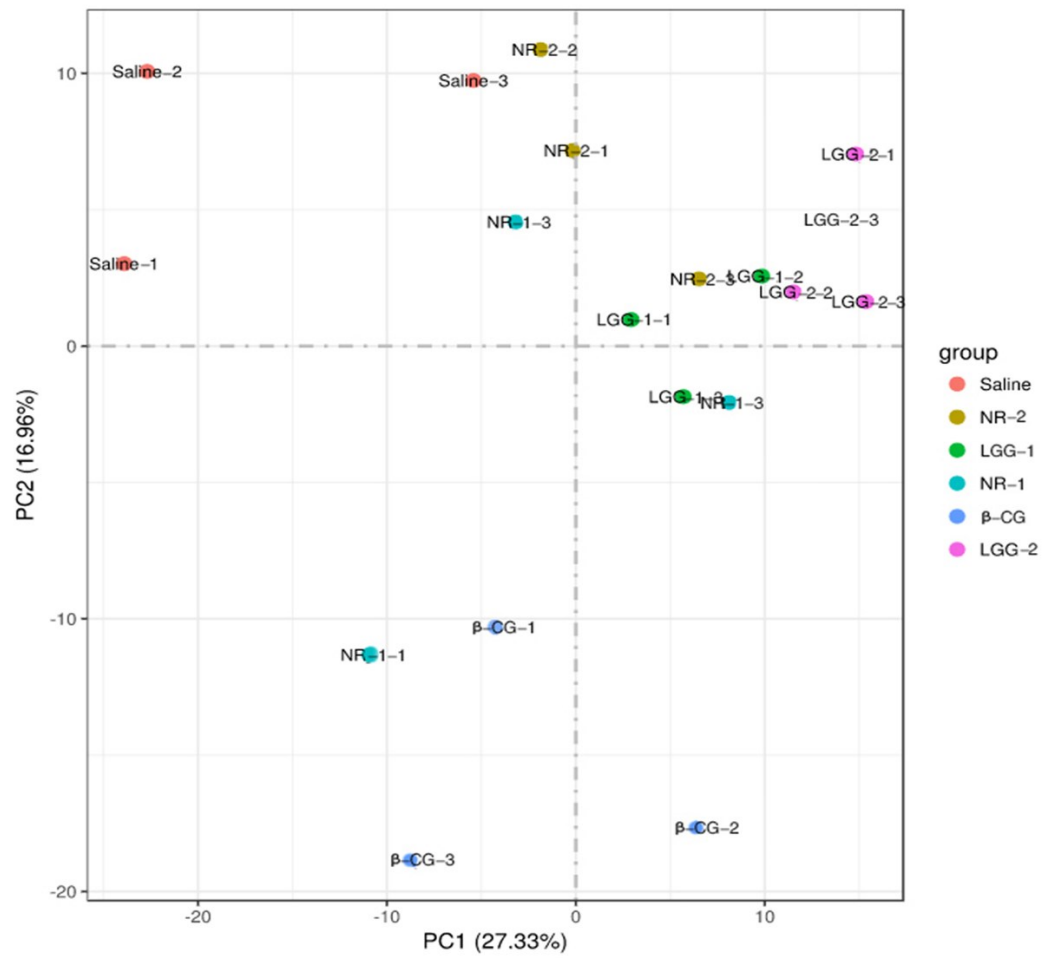


Figure S3 Score plots (PC1 × PC2) of saline group, β-CG group, LGG-1 group, LGG-2 group, NR-1 group and NR-2 group based on the gene expression levels of spleen.