Am J Transl Res 2025;17(8):6333-6346
www.ajtr.org /ISSN:1943-8141/AJTR0165767

Original Article

YiQiWenShen formula ameliorates myasthenia gravis through T follicular helper cells/T follicular regulatory cells immune rebalancing

Kaihua Rao[1], Zhengfeng Li[2], Chunhua Huang[1]

[1]Department of Neurology, Affiliated Hospital of Jiangxi University of Traditional Chinese Medicine, Nanchang 330006, Jiangxi, China; [2]Department of Endocrinology, Affiliated Hospital of Jiangxi University of Traditional Chinese Medicine, Nanchang 330006, Jiangxi, China

Received May 1, 2025; Accepted July 26, 2025; Epub August 15, 2025; Published August 30, 2025

Abstract: Background: Myasthenia gravis (MG), a chronic autoimmune neuromuscular disorder characterized by muscle weakness, remains a therapeutic challenge. This study aimed to evaluate the efficacy of the traditional Chinese herbal formulation YiQiWenShen (YQWS) in comparison with prednisone in a rat model of experimental autoimmune myasthenia gravis (EAMG), with the goal of exploring new therapeutic strategies. Methods: EAMG was induced in rats, who were subsequently treated with different doses of YQWS and prednisone. Clinical severity was assessed using the Baggi scoring system, alongside measurements of body weight, serum acetylcholine receptor antibody (AChR-Ab) levels, and splenic lymphocyte proliferation in response to the rat AChRα97-116 peptide and concanavalin A. Cytokine profiles, proportions of T follicular helper (Tfh) cells, T follicular regulatory (Tfr) cells, and germinal center B (GC)-B cells in lymph nodes were analyzed using enzyme-linked immunosorbent assay and flow cytometry. The expression levels of B-cell lymphoma 6 (Bcl-6) mRNA and protein were analyzed using quantitative real-time polymerase chain reaction and western blot, respectively. Results: After EAMG induction, the rats exhibited significant weight loss, elevated clinical scores, and increased AChR-Ab levels. Both YQWS and prednisone treatments significantly improved body weight and clinical scores, and reduced serum AChR-Ab levels. YQWS also attenuated the increased splenic lymphocyte proliferation. Cytokine dysregulation observed in EAMG rats was partially corrected following YQWS treatment. Furthermore, both YQWS and prednisone effectively normalized the Tfh/Tfr cell ratio, decreased GC-B cell populations, and downregulated Bcl-6 expression. Conclusion: YQWS exerts therapeutic effects in EAMG comparable to those of prednisone, demonstrating its potential as an alternative treatment for MG. Its efficacy is associated with the normalization of body weight, modulation of immune responses, correction of cytokine imbalances and reestablishment of Tfh/Tfr and GC-B cell homeostasis.

Keywords: Myasthenia gravis, AChR, Tfh/Tfr, YiQiWenShen formula, Bcl-6

Introduction

Myasthenia gravis (MG) is an autoimmune disease characterized by muscle weakness and fatigue resulting from impaired neuromuscular transmission. The disease primarily affects young women and older men, with an estimated prevalence of approximately 12 per 100,000 individuals [1]. MG is predominantly caused by autoimmune disruption at the neuromuscular junction (NMJ), most commonly through the production of autoantibodies directed against acetylcholine receptors (AChRs). The pathogenesis of MG involves a multifactorial interplay between genetic predisposition and environmental triggers, ultimately leading to a loss of immune tolerance and the generation of pathogenic autoantibodies [2]. Current therapeutic strategies include plasma exchange, intravenous immunoglobulin, immunoadsorption, immunosuppressive agents, targeted immunotherapy and thymectomy. Although these methods can alleviate clinical symptoms, they are not curative [3]. Prednisone is widely used in the treatment of MG due to its anti-inflammatory and immunosuppressive properties. However, its long-term use is associated with significant adverse effects,

https://doi.org/10.62347/CYDE1809

including osteoporosis, hypertension, diabetes and an increased susceptibility to infection, which can limit its clinical use [4]. As a result, there is increasing interest in the development of alternative therapies that offer more selective immunomodulation with improved safety profiles.

Traditional Chinese Medicine (TCM) has garnered increasing attention for its therapeutic efficacy, multi-target mechanisms, and relatively low incidence of adverse effects [5]. Chinese herbal formulations have demonstrated the potential to regulate immune responses while mitigating the risks associated with systemic immunosuppressive therapies, such as those seen with long-term prednisone use [6]. A representative example is the Jianpi Yiqi Bugan Yishen Decoction, which has shown promise in clinical applications [7]. According to TCM theory, MG is primarily associated with "spleen Qi deficiency and impairs the ascent of clear yang". Therapeutic strategies that focus on tonifying Qi and strengthening kidney function are believed to support immune regulation and restore internal balance [8], thereby addressing the underlying pathophysiology of autoimmune diseases such as MG [9]. YiQiWenShen (YQWS) is a traditional herbal formula specifically designed to invigorate the spleen and kidneys while elevating Qi. It has been reported to exert beneficial effects in regional clinical settings in China. Rooted in TCM theories, YQWS embodies the holistic view of disease by emphasizing the interconnection among internal organs, Qi (vital energy), and environmental influences [10]. The constituent herbs of YQWS have long been used for their roles in Qi invigoration and kidney warming, and are thought to contribute to immune homeostasis and systemic vitality. The therapeutic rationale for using YQWS in MG lies in its immunomodulatory potential rather than broad suppression. By correcting immune imbalance and reducing autoantibody production, YQWS offers a targeted approach that may provide symptom relief while minimizing side effects.

Cell-mediated immunity, particularly the regulation of T lymphocyte subsets, has emerged as a crucial target in the treatment of MG, as it plays a central role in B-cell activation and subsequent autoantibody production [11]. Among these subsets, T follicular helper (Tfh) cells/T follicular regulatory (Tfr) cells play key roles in regulating antibody production by B cells [12]. Dysregulation of Tfh/Tfr cell populations and their associated cytokines, such as interferon gamma (IFN-γ), interleukin (IL)-4, IL-6, IL-17, and transforming growth factor beta (TGF-β), can precipitate the autoimmune response characteristic of MG [13, 14]. Consequently, therapeutic strategies aimed at modulating these immune elements may represent promising avenues for intervention. B-cell lymphoma 6 protein (Bcl-6), a transcription factor essential for the development of both Tfh and germinal center B (GC-B) cells, serves as a valuable biomarker for assessing immune activation and the efficacy of immunomodulatory treatments [15]. In parallel, splenic lymphocyte proliferation assays in response to specific antigens, such as the Rat AChRα97-116 peptide (R97-116) or mitogens like concanavalin A (ConA), are employed to evaluate antigen-specific and polyclonal T-cell response, respectively. R97-116, derived from the acetylcholine receptor (AChR), induces lymphocyte proliferation, indicating an aggressive autoimmune response and reflecting disease activity in experimental autoimmune myasthenia gravis (EAMG) [16]. In contrast, ConA, which binds specific sugar molecules on lymphocyte surfaces, serves as a potent nonspecific T-cell mitogen [17]. Notably, in thymic tissues of MG patients, Tfh cells and B cells co-localize within ectopic germinal centers (GCs), where their interactions are thought to drive the generation of pathogenic anti-AChR antibodies [18]. Studies have shown that decreasing the proportions of Th1, Th17, and Tfh cells while increasing Th2, Treg, and Tfr populations can significantly alleviate MG symptoms [19]. Therefore, restoring the balance of T-cell subsets may constitute a key immunomodulatory mechanism in MG therapy. Targeting Tfh/Tfr cells may hold promise for the development of more effective therapeutic approaches for MG.

This study aims to systematically evaluate the therapeutic efficacy of the traditional Chinese medicine formula YQWS in a rat model of experimental autoimmune myasthenia gravis (EAMG), and to elucidate the molecular mechanisms underlying its immunomodulatory effects. By directly comparing its outcomes with those of the standard therapy, Prednisone,

we seek to investigate the relative advantages of YQWS in terms of safety and immune regulation, thereby exploring novel therapeutic avenues and safer management strategies for MG.

Materials and methods

Materials

The YQWS formula was meticulously devised by the authors based on traditional Chinese medicine theory and comprised of the following components: *Astragalus membranaceus* (60 g), *Codonopsis pilosula* (30 g), *Tangerine Peel* (10 g), *Cimicifugae Rhizoma* (10 g), *Bupleuri Radix* (10 g), *Atractylodes macrocephala Koidz* (15 g), *Prepared Liquorice Root* (10 g), *Angelica sinensis* (15 g), *Cuscuta chinensis* (30 g), *Goji Berries* (30 g), *Cornus officinalis* (20 g), and *Deer Antler* (10 g). Prednisone acetate tablets (5 mg) were obtained from Tianjin Tianshili Pharmaceutical Co., Ltd., China (National Drug Approval No. H12020689). The R97-116 peptide (DGDFAIVK-FTKVLLDYTGHI) was procured from Suzhou Genscript Biotech Co., Ltd. Complete Freund's adjuvant (CFA) and concanavalin A (ConA) were procured from Sigma-Aldrich, USA. The following antibodies were sourced from Invitrogen (Thermo Fisher Scientific, Inc., MA, USA): fluorescein isothiocyanate (FITC)-labeled anti-rat CD4 (11-0040-82), PE/Cy7-labeled anti-rat inducible T-cell costimulator (ICOS; 25-9949-82), and allophycocyanin (APC)-labeled anti-rat forkhead box P3 (Foxp3; 17-5773-82). PE-labeled anti-rat C-X-C motif chemokine receptor 5 (CXCR5; 551959) and Mycobacterium tuberculosis H37Ra lyophilized powder from BD Biosciences (CA, USA) were utilized. Enzyme-linked immunosorbent assay (ELISA) kits for IFN-γ, IL-4, IL-6, IL-17, and TGF-β were obtained from eBioscience (CA, USA). Biotinylated peanut agglutinin (PNA) was obtained from Vector Laboratories (CA, USA). The Cell Counting Kit-8 (CCK-8) was procured from Dojindo Molecular Technologies, while primers for real-time quantitative polymerase chain reaction (RT-qPCR) were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Antibodies for Bcl-6 (ab-33901), AChR (ab259859), Cy3-labeled donkey anti-goat (ab6949), β-actin (ab213262), and goat anti-rabbit IgG H&L (HRP) (ab2057-18), were all sourced from Abcam (Cambridge, UK). Reagents for protein analysis, including sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), bovine serum albumin (BSA), and tris-buffered saline with Tween 20 (TBST) buffer, were acquired from Solarbio (Beijing, China). The PrimeScript RT reagent kit and SYBR® Premix Ex TaqTM II kit from Takara Bio, Japan.

Experimental animals

Following a one-week acclimation period, 120 specific pathogen-free (SPF) female Lewis rats (6-8 weeks old, weighing 160-180 g) were kept in the SPF barrier facility of the Experimental Animal Center at Fujian Anburui Biotechnology Co., Ltd. The animals were randomly divided into six subgroups (n = 20 per group). Rats were housed in polystyrene cages under controlled environmental conditions, including a 12/12 hour light/dark cycle, a temperature of 24±1°C, and a humidity of 50%±12%, in compliance with the guidelines of the National Institutes of Health. All animals had *ad libitum* access to standard laboratory chow and water. All experimental procedures involving animals were approved by the Ethical Committee of Fujian Anburui Biotechnology Co., Ltd. (Approval No. IACUC FJABR 2022026032).

Modeling and clinical assessment

The EAMG rat model was established according to the protocol described by Baggi et al. [20]. On day 0, a 200-μL emulsion of CFA containing 50 μg of the R97-116 peptide and 1 mg of H37RA dry powder was prepared and administered at four sites: the base of the tail, both hind footpads, and bilaterally on the back. Rats in the adjuvant control group received an equal volume of CFA mixed with phosphate-buffered saline (PBS). On days 30 and 45, booster immunizations were administered at the same injection sites and in the same volume, using incomplete Freund's adjuvant (IFA) emulsified with 50 μg of the R97-116 peptide. The adjuvant control group received IFA mixed with PBS emulsion. Buprenorphine (0.05 mg/kg) was administered 30 min prior to the surgical procedures to ensure preemptive analgesia. Successful model establishment was confirmed based on the presence of all three of the following criteria: (1) significant weight loss, (2) clinical symptom score > 1 based on the Baggi scale, and (3) increased serum AChR-Ab levels. Model evaluation was conducted seven

days after the second immunization to ascertain that 100 rats satisfied the EAMG criteria. These EAMG rats were then randomly divided into the following groups (n = 20 per group): model group, low-dose YQWS group (4 g/kg/day), medium-dose YQWS group (8 g/kg/day), and high-dose YQWS group (16 g/kg/day), and a positive control group (Prednisone, 5.4 mg/kg/day). All treatments were administered by oral gavage for 30 consecutive days. Clinical severity was assessed using the Baggi scoring system, which ranges from 0 to 4. A score of 0 indicates normal muscle strength; 1 denotes mild fatigue, weak grip strength, and decreased activity; 2 represents moderate symptoms including significant reduction in activity, noticeable weight loss, hunched posture, drooping head, flexed forearms, and tremors; 3 indicates severe generalized weakness with complete loss of grip strength and inability to maintain posture; and 4 corresponds to death. Intermediate symptoms were scored as 0.5, 1.5, and 2.5, respectively. Clinical assessments were performed prior to treatment, as well as at 2 and 4 weeks after the initiation of treatment. Body weight was recorded every 5 days throughout the treatment period. At the end of the experiment, euthanasia was performed by intraperitoneal injection of pentobarbital sodium (150 mg/kg), followed by bilateral thoracotomy to confirm death.

AChR-Ab analysis

Serum samples were collected from the tail vein (7 days after the second immunization) and from the abdominal aorta at the experimental endpoint. Samples were centrifuged at 3000×*g* for 10 min at 4°C to separate serum. AChR-Ab concentrations were quantified using a competitive ELISA kit (Sangon Biotech) following the manufacturer's specifications. Briefly, serum samples were further centrifuged at 10,000×*g* for 10 min to remove any precipitates. After allowing all reagents to equilibrate to room temperature (21°C) for 20 minutes, standards and samples were incubated for 45 min at 37°C in antigen-precoated wells with biotin-conjugated antibody. Following five washes with PBS, HRP-streptavidin conjugate was added and incubated for 30 min at 37°C. After repeated washing, tetramethylbenzidine (TMB) substrate reaction was added for color development (15 min at 37°C, protected from light). The reaction was terminated with acidic solution, and absorbance was measured at 450 nm within 5 min.

Preparation of spleen lymphocyte suspension

Following the treatment period, rats were anesthetized, and the abdominal region was disinfected with ethanol. A small incision was made on the left side of the abdomen using ophthalmic scissors. The connective tissues surrounding the spleen were meticulously dissected, and the spleen was excised under sterile conditions. The extracted spleen was placed in a culture dish and minced into small fragments. The tissue was then gently homogenized using the plunger of a 5-ml syringe over a 200-mesh nylon screen cell strainer to obtain a single-cell suspension. The suspension was collected and centrifuged, and the resulting cell pellet was washed twice with PBS to yield purified splenic lymphocytes for subsequent analyses.

CCK8 assay

A single cell suspension of splenic lymphocytes was prepared and seeded at a density of 3×10^4 cells per well in a 96-well plate. Cells were cultured in RPMI 1640 medium and stimulated with either 5 μg/mL concanavalin A (ConA) or 10 μg/mL R97-116 peptide for 72 hours at 37°C, as previously described [21, 22]. The control group was the standard medium. Subsequently, 10 μL of CCK-8 reagent was added to each well, followed by incubation at 37°C for 1 hour. Cell proliferation was assessed by measuring the optical density (OD) at 450 nm using a microplate reader.

ELISA

Following euthanasia, thymic tissues were carefully excised and homogenized using a homogenizer to obtain a uniform suspension. The homogenates were centrifuged at 3000×*g* for 15 min to collect the supernatants, which were then added to the wells to allow specific binding to IFN-γ, IL-4, IL-6, IL-17, and TGF-β antigens. Subsequently, enzyme-conjugated secondary antibodies were applied to detect the antigen-antibody complexes. The addition of substrate solution produced a color change proportional to the cytokine concentration. The optical density was measured using a spectrophotometer, with cytokine levels calculated based on a standard curve generated from known cytokine concentrations.

Flow cytometry analysis

For the identification of Tfh cells, splenic lymphocytes (1×10^5 cells) were stained with 1 μL each of anti-CD4-FITC, anti-CXCR5-PE, and anti-ICOS-PE/Cy7. An additional 5 μL of anti-Foxp3-PE was incorporated for the analysis of Tfr cells. After staining, cells were washed and examined using a flow cytometer after a 30-min culture period at 4°C in the dark. The findings were processed using FlowJo software V10.

Lymph node PNA staining

Frozen lymph node sections (7 μm thickness) were subjected to peanut agglutinin (PNA) staining to identify germinal centers. Sections were first treated with 0.3% H_2O_2 in methanol for 15 min to quench endogenous peroxidase activity. After blocking with 5% normal horse serum for 30 min, sections were incubated with biotinylated PNA (1:500 dilution) for 1 h at room temperature. Following three 2-min washes with TBST, nuclei were counterstained using a mounting medium containing 4',6-diamidino-2-phenylindole (DAPI) for 5 min. PNA binding was visualized using fluorescence microscopy.

Neuromuscular junction (NMJ) AChR immunofluorescence staining

Transverse sections of rat skeletal muscle were fixed in cold acetone for 15 min at 4°C. Sections were permeabilized with 1 g/l Triton X-100 for 30 min, followed by thorough washing with PBS. Non-specific binding sites were blocked using 50 ml/l serum for 1 h at room temperature. The sections were then incubated with goat anti-rabbit AChR primary antibody (1:100 dilution) at 37°C for 2 h. Following washing, the primary antibody was detected with Cy3-conjugated donkey anti-goat secondary antibody (1:200 dilution) at 21°C for 2 h. After final PBS washes, nuclei were stained with DAPI-containing mounting medium for 5 min. AChR localization distribution was assessed by fluorescence microscopy.

RT-qPCR

Total RNA was isolated from rat thymus tissue using TRIzol reagent. Following phase separation, RNA pellet was collected by centrifugation at 12,000×*g* for 10 min at 4°C, washed, and dissolved in 10 μL of DEPC-treated water. Complementary DNA synthesis was performed using the PrimeScript RT kit. Quantitative real-time PCR (RT-qPCR) was then carried out with the SYBR® Premix Ex TaqTM II kit on an Applied Biosystems®7500 Real-Time PCR System (USA). The PCR was conducted under the following conditions: initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 32 s. Relative expression levels of Bcl-6 mRNA were calculated using the $2^{-\Delta\Delta Ct}$ method [23], with β-actin serving as the internal reference gene. The primer sequences used were: Bcl-6 F: ATCGAGGTCGTGAGGTTGTG, R: TCGGATAAGAGGCTGGTGG; β-actin F: AGGAGTACGATGAGTCCGGC, R: AAGAAAGGGTGTAAAACGCAGC.

Western blot

Thymus tissues were lysed in RIPA buffer, and protein concentrations were quantified using a bicinchoninic acid assay kit. Equal amounts of denatured proteins were separated by 10% SDS-PAGE and transferred onto polyvinylidene fluoride membranes. Membranes were blocked with 5% bovine serum albumin (BSA), and incubated overnight at 4°C with primary antibodies against Bcl-6 (1:500) and β-actin (1:1,000). The sections were then incubated with goat anti-rabbit antibody IgG H&L (HRP) (1:20,000) for two hours at 25°C after being washed twice with TBST solution (0.05% Tween 20 in TBS) for 15 minutes. As a loading control, anti-β-actin antibody (1:5,000) was used.

Data analysis

Experimental animals were randomly assigned to groups to minimize bias. Data processing and statistical analyses were conducted using SPSS version 20.0 software. Data conforming to a normal distribution were expressed as the mean ± standard deviation. Homogeneity of variance was first assessed for comparisons of quantitative data between groups. When variances were equal, one-way analysis of variance (ANOVA) was employed. For data exhibiting unequal variances, non-parametric tests were employed as an alternative. A two-sided *p*-value of less than 0.05 was considered significant.

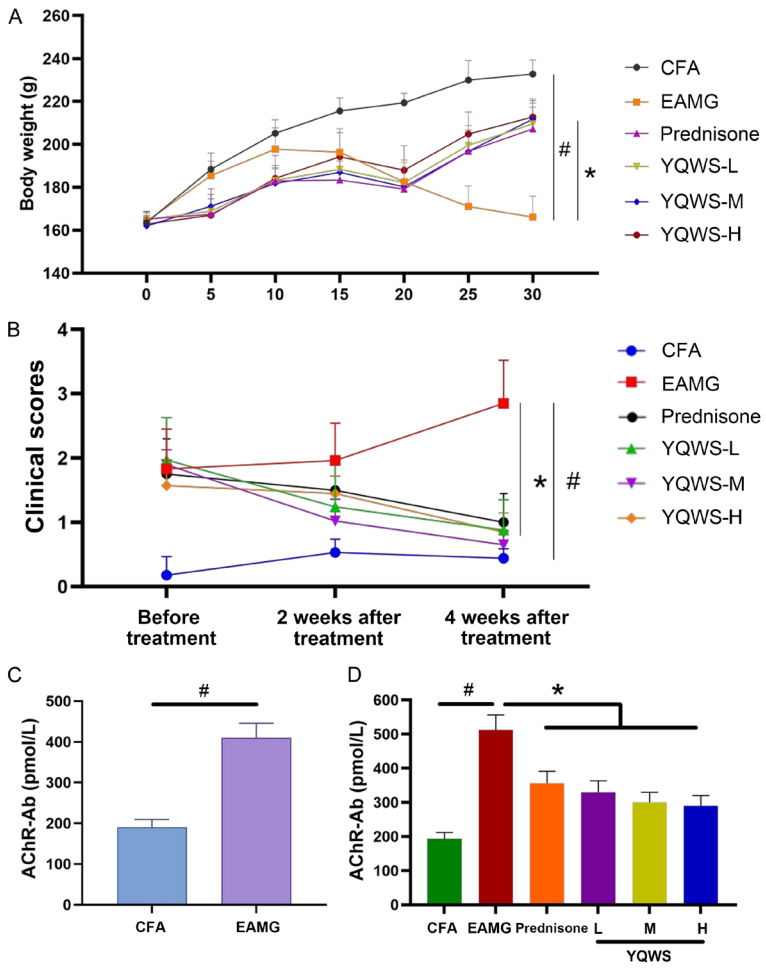

Figure 1. Changes in body weight and clinical scores in EAMG rats. This figure illustrates the effects of YQWS and Prednisone on body weight and clinical scores in EAMG rats. A. Graph showing the trend in body weight from day 15 to day 30 post-induction, comparing the control group with treatment groups. B. Graph showing the changes in clinical scores of EAMG rats after 2 and 4 weeks of treatment, highlighting the medium dose's effectiveness. C. Bar graph showing AChR-Ab levels in the serum of EAMG rats. D. Bar graph showing AChR-Ab levels in the serum of EAMG rats after treatment with Prednisone or YQWS. CFA, Prednisone, YQWS low (L), medium (M), and high (H) dose (n = 20 per group). EAMG group (n = 18). #, *P < 0.05. Abbreviations: EAMG, Experimental Autoimmune Myasthenia Gravis; YQWS, YiQi-WenShen formula; CFA, Complete Freund's Adjuvant; AChR-Ab, Acetylcholine receptor antibodies.

Results

Weight and clinical symptom improvements in EAMG rats

Compared to the CFA group, EAMG-induced rats exhibited a significant decrease in body weight starting from day 15 post-induction, which persisted through day 30. Treatment with varying doses of the YQWS formula, as well as the Prednisone positive control, significantly improved body weight in EAMG rats ($P < 0.05$), as depicted in **Figure 1A**. No statistically significant differences in weight gain were observed among the various treatment doses (detailed data are presented in **Table 1**). According to the Baggi clinical symptom grading scale, all EAMG-model rats scored > 1, confirming successful model establishment (**Table 2**), while the CFA group maintained a healthy status with scores of 0.18±0.29. Post-treatment evaluations at 2 and 4 weeks revealed a significant reduction in clinical scores in EAMG rats (**Figure 1B**), with the medium-dose YQWS group showing the greatest improvement at both time points. Notably, all YQWS doses induced a more substantial decrease in clinical scores in comparison to Prednisone treatment ($P < 0.05$) (**Table 2**). Furthermore, serum AChR-Ab levels in EAMG rats were significantly elevated prior to treatment relative to the CFA controls ($P < 0.05$) (**Figure 1C**). Subsequent to treatment, AChR-Ab levels markedly decreased in rats receiving YQWS at various dosages as well as Prednisone, compared to the EAMG group ($P < 0.05$) (**Figure 1D**). These findings confirm the successful induction of EAMG and demonstrate the therapeutic efficacy of the YQWS formula.

Effect of YQWS on AChR expression at the NMJ

Given the significant reduction in AChR-Ab levels observed with YQWS treatment in EAMG rats, we further examined AChR expression at the NMJ. EAMG rats exhibited an autoimmune response, characterized by antibody-induced AChR degradation, resulting in decreased AChR

Table 1. Changes in body weight (g) of rats in each group

Group	n	0 d	5 d	10 d	15 d	20 d	25 d	30 d
CFA	20	163.6±2.1	188.4±7.6	205.2±6.2	215.6±6.1	219.4±4.5	230.0±9.0	232.8±6.5
EAMG	18	164.1±3.9	185.4±6.8	197.8±9.9	196.4±10.9	182.5±10.2	171.0±9.6	166.1±9.8
Prednisone	20	162.8±4.1	167.0±9.6	184.2±10.6	194.2±11.3	188.0±11.4	204.8±10.3	212.8±8.4
YQWS-L	20	165.2±3.6	167.4±7.3	183.0±6.1	183.4±8.8	179.2±9.3	196.8±9.9	207.2±10.1
YQWS-M	20	165.0±3.2	168.8±5.8	183.2±5.4	188.4±6.7	182.2±9.3	199.6±9.2	209.6±9.5
YQWS-H	20	162.0±6.7	171.2±8.1	181.8±8.4	187.0±9.3	180.2±8.2	196.8±7.9	211.8±8.6

Abbreviations: EAMG, Experimental Autoimmune Myasthenia Gravis; CFA, Complete Freund's Adjuvant; YQWS, YiQiWenShen formula; d, day.

Table 2. Effects of YQWS on clinical scores in rats

Group	n	Before treatment	2 weeks after treatment	4 weeks after treatment
CFA	20	0.18±0.29	0.53±0.21	0.44±0.15
EAMG	18	1.83±0.62	1.96±0.58	2.85±0.67
Prednisone	20	1.75±0.55	1.50±0.42	1.00±0.45
YQWS-L	20	1.97±0.66	1.24±0.65	0.88±0.47
YQWS-M	20	1.90±0.23	1.02±0.34	0.65±0.22
YQWS-H	20	1.57±0.22	1.45±0.27	0.85±0.3

Abbreviations: EAMG, Experimental Autoimmune Myasthenia Gravis; CFA, Complete Freund's Adjuvant; YQWS, YiQiWenShen formula.

density and structural abnormalities at the NMJ. These changes reflect the pathologic impact on muscle fibers and nerve terminals inherent to the disease. Treatment with either YQWS or Prednisone significantly increased AChR expression, with the medium dose of YQWS yielding the most pronounced effect (**Figure 2**). Notably, while prednisone increased AChR expression, it did not significantly improve NMJ structural integrity, which was significantly ameliorated by YQWS across all tested doses.

Lymphocyte proliferation response to YQWS treatment

Splenic lymphocytes isolated from normal and EAMG rats were stimulated with 10 μg/mL R97-116 peptide or 5 μg/mL ConA. The CCK8 assay indicated an increased proliferation of splenic lymphocytes from EAMG rats upon R97-116 or ConA stimulation compared to cells cultured in RPMI 1640 medium. This hyperproliferative response was partially reversed by treatment with YQWS (25 μM) (**Figure 3**). These findings suggest that YQWS may modulate immune responses, contributing to its beneficial effects on the clinical manifestations of EAMG.

Cytokine profile modulation in EAMG rats

Cytokine levels in thymus tissue were assessed to evaluate immune alterations in EAMG rats. ELISA results demonstrated a significant upregulation of IFN-γ, IL-4, IL-6, IL-17, alongside a decrease in TGF-β compared to the CFA control group ($P < 0.05$). Treatment with the YQWS formula or Prednisone partially offset these immunological imbalances (**Figure 4**), with medium and high doses of the YQWS showing similar efficacy comparable to Prednisone (**Table 3**).

Regulation of Tfh/Tfr cell proportions by YQWS

Analysis of Tfh/Tfr immune cell phenotypes revealed a significant increase in the proportion of Tfh cells in EAMG rats compared to the CFA group. Administration of YQWS or Prednisone reduced Tfh cell proportions, with the high-dose YQWS group achieving effects comparable to Prednisone (**Figure 5A**). Conversely, the proportion of Tfr cells, which was decreased in EAMG rats, was increased following treatment with both YQWS and Prednisone, with the formula's effects paralleling those of the high-dose group (**Figure 5B**). These findings corroborate the role of the YQWS in rebalancing Tfh/Tfr immune cells as part of its therapeutic effect on EAMG.

Impact of YQWS on GC-B cells and Bcl-6 expression

Given the pivotal roles of Tfh and Tfr cells in regulating GC-B cell function, PNA staining was

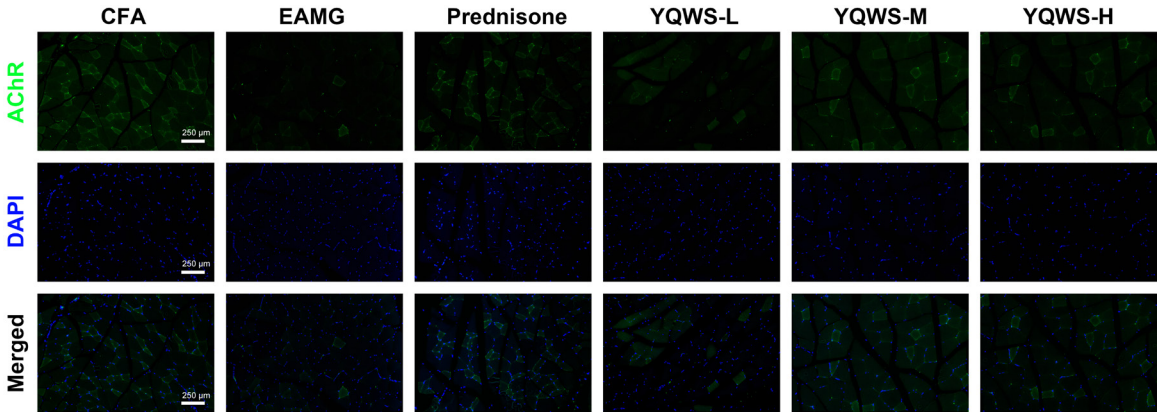

Figure 2. Immunofluorescence analysis of YQWS effects on AChR expression at the NMJ in EAMG rats. Scale bar = 250 μm, magnification: 200×, n = 6. Abbreviations: CFA, Complete Freund's Adjuvant; DAPI, 4',6-diamidino-2-phenylindole; IF, immunofluorescence; NMJ, Neuromuscular junction; YQWS, YiQiWenShen formula; AChR, Acetylcholine receptor; EAMG, Experimental Autoimmune Myasthenia Gravis.

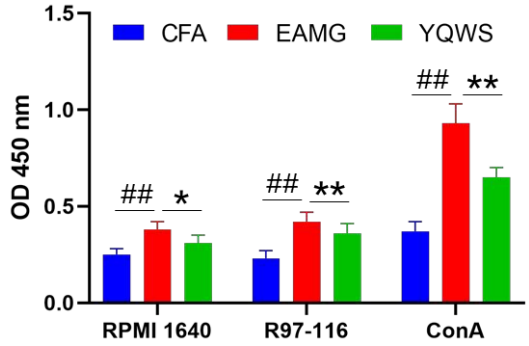

Figure 3. Effect of YQWS on splenic lymphocyte proliferation in EAMG rats. CCK8 assay results indicating the proliferation of splenic lymphocytes from EAMG rats stimulated with R97-116 or ConA, with or without YQWS treatment. n = 6. *P < 0.05, ##, **P < 0.01. Abbreviations: EAMG, Experimental Autoimmune Myasthenia Gravis; CFA, Complete Freund's Adjuvant; OD, optical density; CCK8, Cell Counting Kit-8; YQWS, YiQiWenShen formula; R97-116, peptide stimulation; ConA, Concanavalin A.

employed to evaluate GC-B cell proportions in the lymph nodes of EAMG rats. Results demonstrated an increase in GC-B cells in EAMG rats compared to CFA controls, which was reduced following treatment with YQWS and prednisone, with the medium dose of YQWS exhibiting the most pronounced effect (**Figure 6A**). A comparison of the results obtained from the present study with those from CFA controls revealed that EAMG rats elevated mRNA and protein expression levels of Bcl-6, a master transcription factor essential for GC-B and Tfh cell production. Treatment with either YQWS or prednisone significantly attenuated these elevations, with elevated doses of YQWS exerting effects comparable to prednisone (**Figure 6B**, **6C**).

Discussion

Myesthenia Gravis (MG) primarily results from the immune system producing antibodies that block or destroy acetylcholine receptors at the NMJ, disrupting normal neuromuscular transmission. This interference impairs muscle contraction, resulting in muscle weakness [24]. While conventional treatments have enabled many patients to achieve normal or near-normal life, disease monitoring and management remain a lifelong challenge [25]. In this context, this study explores the therapeutic effects of the YQWS herbal formula on EAMG in rats, aiming to elucidate its underlying mechanism of action.

The observed improvement in body weight among YQWS-treated EAMG rats serves as a key indicator of restored overall health and mitigation of muscle atrophy. This enhancement likely reflects reduced systemic inflammation and recovery of muscle strength, both critical for maintaining organismal vitality [26]. However, the lack of substantial differences in weight gain across different YQWS dosages suggests a threshold effect, whereby doses beyond a certain level confer no additional benefit. The concurrent decrease in clinical symptom scores further confirms the formula's effi-

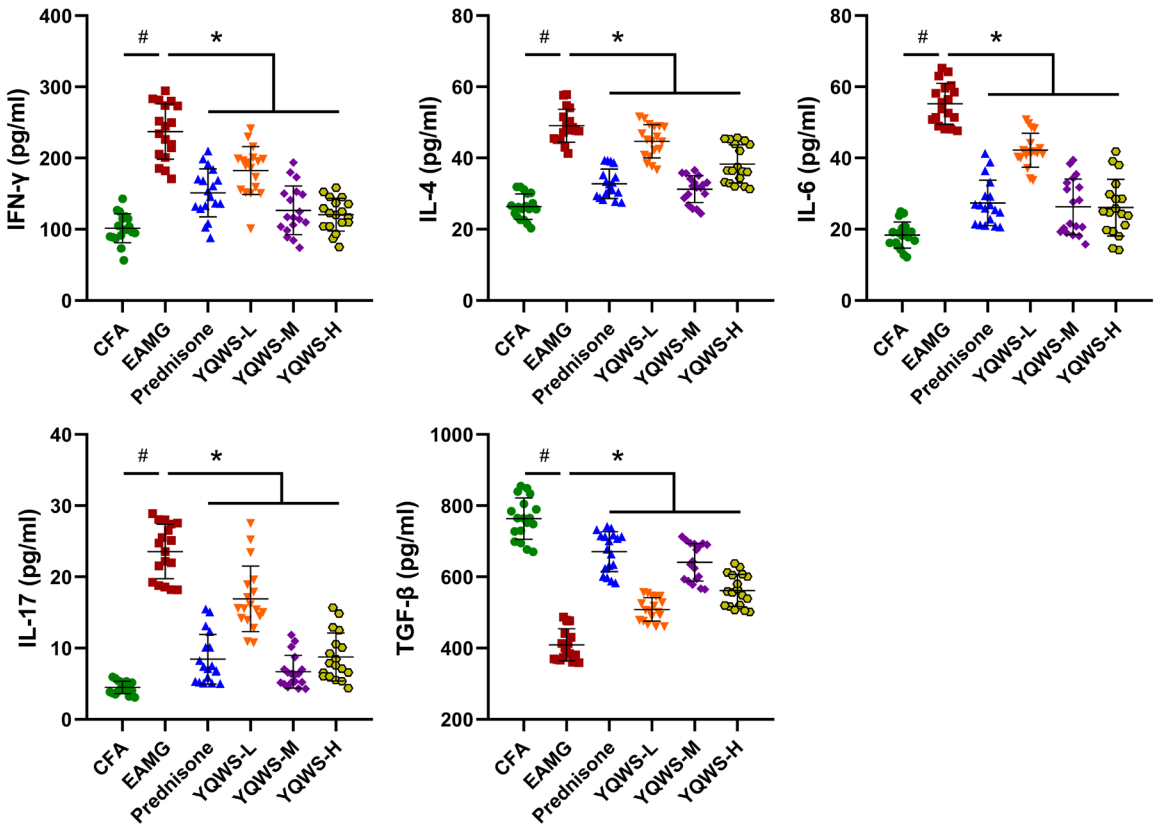

Figure 4. Modulation of cytokine profile in EAMG rats following YQWS and prednisone treatment. A. ELISA results showing levels of IFN-γ, IL-4, IL-6, IL-17, and TGF-β in EAMG compared to CFA controls. B. Comparative analysis of cytokine levels after treatment with YQWS and Prednisone. n = 18. #, *P < 0.05. Abbreviations: EAMG, Experimental Autoimmune Myasthenia Gravis; CFA, Complete Freund's Adjuvant; ELISA, Enzyme-Linked Immunosorbent Assay; IFN, Interferon; YQWS, YiQiWenShen formula; IL, Interleukin; TGF-β, Transforming Growth Factor Beta.

Table 3. Effects of YQWS on cytokine levels (pg/ml) in rat thymus

Group	n	IFN-γ	IL-4	IL-6	IL-17	TGF-β
CFA	18	101.6±20.4	26.3±3.6	18.4±3.7	4.5±0.9	763.9±57.9
EAMG	18	237.3±38.8	49.1±4.6	55.2±5.8	23.6±3.8	409.3±45.0
Prednisone	18	151.0±33.7	32.8±4.1	27.4±6.4	8.4±3.5	671.2±56.0
YQWS-L	18	182.6±33.6	44.7±4.6	42.2±4.8	16.9±4.6	508.6±33.0
YQWS-M	18	126.6±34.1	31.3±3.8	26.3±7.8	6.7±2.3	641.0±52.7
YQWS-H	18	120.6±23.0	38.3±5.4	26.1±8.0	8.7±3.4	561.8±45.1

Abbreviations: EAMG, Experimental Autoimmune Myasthenia Gravis; CFA, Complete Freund's Adjuvant; YQWS, YiQiWenShen formula; IFN, Interferon; YQWS, YiQiWenShen formula; IL, Interleukin; TGF-β, Transforming Growth Factor Beta.

cacy, demonstrating alleviation of hallmark EAMG manifestations such as muscle weakness and fatigue. These clinical improvements suggest not only symptomatic relief but possibly a restoration of neuromuscular function - an effect that may surpass that typically achieved by conventional treatments such as prednisone.

AChR-Ab are central to the pathogenesis of MG, where they disrupt neuromuscular transmission and induce muscle weakness [27]. Our data show that YQWS treatment significantly reduces AChR-Ab levels, highlighting the formula's ability to target the core autoimmune mechanism in EAMG [28]. This conclusion is further supported by immunofluorescence analysis of

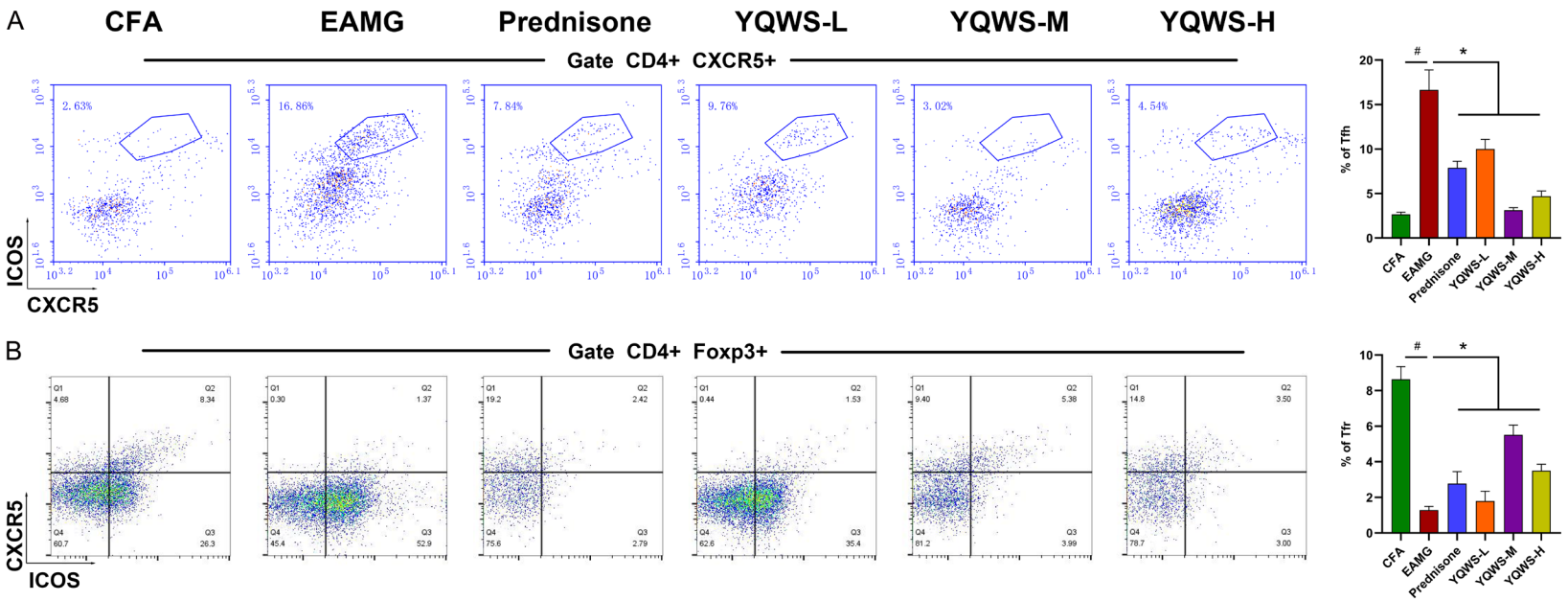

Figure 5. Regulation of Tfh/Tfr cell proportions by YQWS treatment. This figure depicted the alterations in Tfh/Tfr cell proportions in EAMG rats treated with YQWS and Prednisone. A. Graphical representation of Tfh cell proportions in EAMG rats following treatment with YQWS and Prednisone compared to controls. B. Analysis of Tfr cell proportions post-treatment, showing the effects of YQWS and Prednisone. n = 6. #, *P < 0.05. Abbreviations: EAMG, Experimental Autoimmune Myasthenia Gravis; CFA, Complete Freund's Adjuvant; CXCR5, C-X-C motif chemokine receptor 5; ICOS, Inducible costimulator; Foxp3, forkhead box P3; YQWS, YiQiWenShen formula; Tfh, T follicular helper; Tfr, T follicular regulatory.

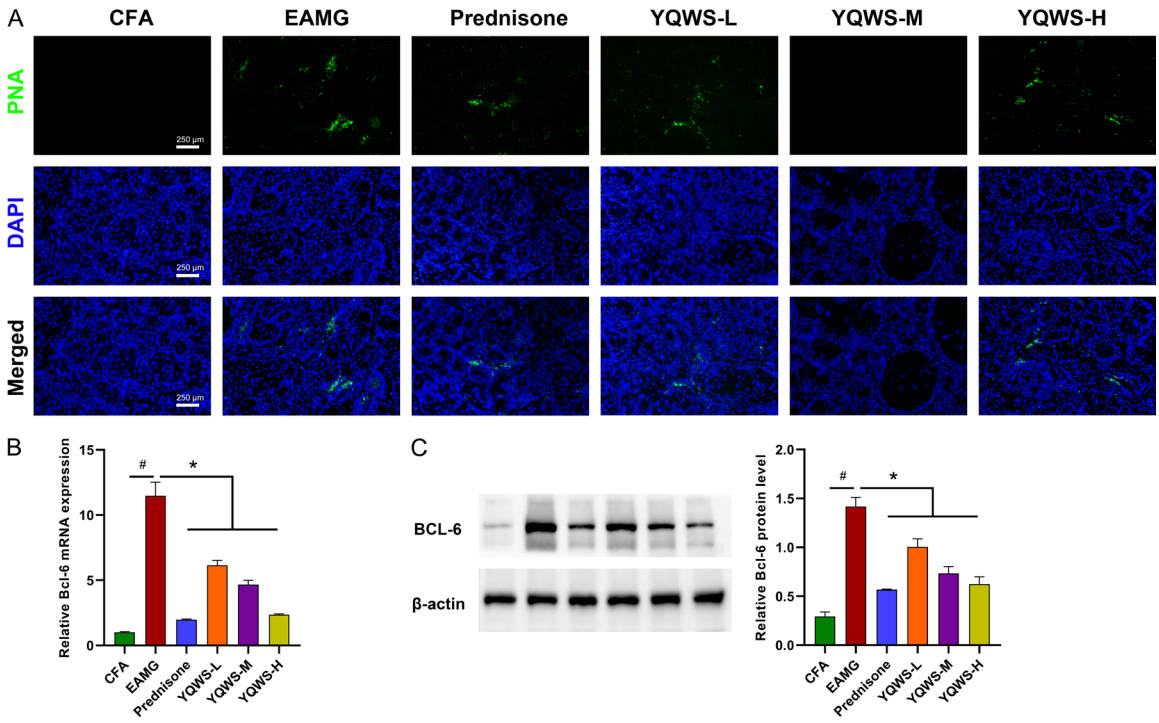

Figure 6. Effects of YQWS on GC-B cells and Bcl-6 expression in EAMG rats. (A) PNA staining results indicating changes in GC-B cell proportions in lymph nodes. (B) Graphical representation of mRNA levels and (C) protein expression of Bcl-6 pre- and post-treatment. Scale bar = 250 μm, magnification: 200×, n = 3. #, *P < 0.05. Abbreviations: EAMG, Experimental Autoimmune Myasthenia Gravis; CFA, Complete Freund's Adjuvant; DAPI, 4',6-diamidino-2-phenylindole; GC-B, Germinal Center B cells; YQWS, YiQiWenShen formula; PNA, Peanut Agglutinin; mRNA, messenger RNA; Bcl-6, B-cell lymphoma 6.

NMJ AChR expression, which revealed enhanced receptor density after YQWS treatment. Such reductions in AChR-Ab levels typically result from complex immunoregulatory processes [29, 30], suggesting that YQWS exerts modulatory effects on the immune system.

T cells and B cells lie at the core of immune modulation [31]. T cells, which develop in the thymus, recognize specific antigens and orchestrate adaptive immune responses [32]. Our findings suggest that YQWS modulates cellular immunity by partially reversing the heightened proliferation of splenic lymphocytes from EAMG rats stimulated with the R97-116 peptide or ConA, thereby regulating lymphocyte activity. Furthermore, treatment with YQWS or Prednisone partially restored the dysregulated cytokine profiles in EAMG rats, including IFN-γ, IL-4, IL-6, IL-17, and TGF-β-cytokines known to be implicated in MG and other autoimmune diseases [33, 34]. The modulation of these cytokines suggests that YQWS may mediate its therapeutic effects not only by alleviating inflammatory symptoms but also by addressing the underlying autoreactive immune processes characteristic of EAMG, thereby affecting the overall immune response. Notably, higher doses of YQWS demonstrated efficacy comparable to, or exceeding, that of prednisone, suggesting that it may be an alternative therapy for MG.

B cells, which mature in the bone marrow, are responsible for producing antibodies that neutralize pathogens. Tfh cells, a specialized subset of CD4+ T cells residing within B cell follicles, provide essential support to B cells during GC reactions, critical for the generation of effective antibody responses [35]. Conversely, Tfr cells maintain homeostasis within the GC, ensuring that antibody responses remain precise and controlled [36]. This regulatory mechanism is critical for preserving immune tolerance and preventing autoimmune diseases, particularly MG [37]. Our study demonstrates that YQWS treatment decreased Tfh cells while increasing Tfr cells, thereby restoring the Tfh/

Tfr balance. This immunological shift inhibits excessive B cell activation and fosters immune tolerance. Correspondingly, pathogenic autoantibody production was reduced, as evidenced by diminished GC-B cells detected through RNA analysis, indicating reduced GC activity - the site of high-affinity, including autoreactive antibody generation [38]. The observed down-regulation of Bcl-6 expression following YQWS treatment is particularly noteworthy. Given that Bcl-6 acts as a master regulator essential for the differentiation of both Tfh and GC-B cells within the GC [39], its reduction suggests that YQWS (and prednisone) may dampen pathogenic GC dynamics, thereby decreasing autoantibody production and improving disease outcomes.

Our findings highlight advantages of YQWS over traditional treatments like prednisone. While both demonstrate efficacy, the multi-component YQWS formula, grounded in Traditional Chinese Medicine (TCM), likely exerts its effects through a multi-targeted approach. The mechanistic insights of YQWS extend beyond EAMG therapy, suggesting a paradigm shift in autoimmune disease management - from broad immunosuppression toward more refined immune modulation. This shift reflects a deeper understanding of immune complexity and increasing recognition of herbal compounds' capacity for targeted immunoregulatory effects. Notably, the comparable or superior efficacy of higher YQWS doses relative to prednisone supports its potential as a viable alternative for patients who are intolerant or not responsive to conventional immunosuppressive therapies.

In summary, our study provides valuable insight into the therapeutic potential of the YQWS formula in the treatment of EAMG, a well-established animal model of MG. The formula's ability to ameliorate clinical symptoms, reduce AChR-Ab levels, regulate lymphocyte proliferation, restore cytokine profiles, and rebalance Tfh/Tfr and GC-B cell dynamics underscores its promise as an effective multi-targeted treatment option. These findings advance our understanding of MG pathophysiology and immune modulation, highlighting the integration of TCM principles with contemporary therapeutic strategies.

Acknowledgements

This work was supported by 2023 Key Project of Jiangxi Provincial Traditional Chinese Medicine Science and Technology Plan, Jiangxi Provincial Administration of Traditional Chinese Medicine, 2023Z007; The Fifth Batch of National Training Program for Outstanding Clinical Talents in TCM (National TCM People's Education Letter [2022] No. 1); Rao Wangfu National Master's Inheritance Studio (Gan TCM Science and Education Letter [2022] No. 3).

Disclosure of conflict of interest

None.

Abbreviations

AChR-Ab, Acetylcholine Receptor Antibody; CFA, Complete Freund's Adjuvant; ConA, Concanavalin A; CXCR5, C-X-C Motif Chemokine Receptor 5; EAMG, Experimental Autoimmune Myasthenia Gravis; ELISA, Enzyme-Linked Immunosorbent Assay; Foxp3, Forkhead box P3 (transcription factor); GC, Germinal Center; HRP, Horseradish Peroxidase; ICOS, Inducible costimulator; IF, Immunofluorescence; IFN-γ, Interferon Gamma; IFA, Incomplete Freund's Adjuvant; MG, Myasthenia Gravis; PNA, Peanut Agglutinin (germinal center B-cell marker); R97-116, Rat AChRα97-116 peptide (immunogenic epitope); TCM, Traditional Chinese Medicine; Tfh, T Follicular Helper; Tfr, T Follicular Regulatory; TGF-β, Transforming Growth Factor Beta; YQWS, YiQiWenShen Formula.

Address correspondence to: Dr. Chunhua Huang, Department of Neurology, Affiliated Hospital of Jiangxi University of Traditional Chinese Medicine, No. 445, Bayi Avenue, Nanchang 330006, Jiangxi, China. Tel: +86-0791-86363359; E-mail: vich622@126.com

References

[1] Lee HS, Lee HS, Shin HY, Choi YC and Kim SM. The epidemiology of myasthenia gravis in Korea. Yonsei Med J 2016; 57: 419-25.
[2] Gastaldi M, Scaranzin S, Businaro P, Mobilia E, Benedetti L, Pesce G and Franciotta D. Improving laboratory diagnostics in myasthenia gravis. Expert Rev Mol Diagn 2021; 21: 579-590.
[3] Dresser L, Wlodarski R, Rezania K and Soliven B. Myasthenia gravis: epidemiology, patho-

physiology and clinical manifestations. J Clin Med 2021; 10: 2235.
[4] Di L, Shen F, Wen X, Lu Y, Zhu W, Wang M and Da Y. A randomized open-labeled trial of methotrexate as a steroid-sparing agent for patients with generalized myasthenia gravis. Front Immunol 2022; 13: 839075.
[5] Wei D, Yang H, Zhang Y, Zhang X, Wang J, Wu X and Chang J. Nano-traditional Chinese medicine: a promising strategy and its recent advances. J Mater Chem B 2022; 10: 2973-2994.
[6] Zheng S, Xue T, Wang B, Guo H and Liu Q. Chinese medicine in the treatment of ulcerative colitis: the mechanisms of signaling pathway regulations. Am J Chin Med 2022; 50: 1781-1798.
[7] Liu P, Qi G, Gu S, Dong H, Liu C and Yang H. Single-cell transcriptomics and network pharmacology reveal therapeutic targets of Jianpi Yiqi Bugan Yishen decoction in immune cell subsets of children with myasthenia gravis. Transl Pediatr 2022; 11: 1985-2003.
[8] Liu M, Wang H, Lv Z, Lu J, Zhang D, Zhu Z, Wu C, Tian Y, Song C, Wu T, Liu L and Chen X. Invigorating spleen, replenishing qi and tonifying kidney method treatment of traditional Chinese medicine for myasthenia gravis: a protocol for systematic review and meta-analysis. Medicine (Baltimore) 2022; 101: e32285.
[9] Jiang L, Xu P, Zhang D, Lu J, Chang T, Zhang Y and Wang J. Treatment of myasthenia gravis with the method of tonifying spleen and replenishing qi in traditional Chinese medicine: a protocol for systematic review and meta-analysis. Medicine (Baltimore) 2022; 101: e28530.
[10] Li J, Li D, Chen Y, Chen W, Xu J and Gao L. Gut microbiota and aging: traditional Chinese medicine and modern medicine. Clin Interv Aging 2023; 18: 963-986.
[11] Uzawa A, Kuwabara S, Suzuki S, Imai T, Murai H, Ozawa Y, Yasuda M, Nagane Y and Utsugisawa K. Roles of cytokines and T cells in the pathogenesis of myasthenia gravis. Clin Exp Immunol 2021; 203: 366-374.
[12] Ding T, Su R, Wu R, Xue H, Wang Y, Su R, Gao C, Li X and Wang C. Frontiers of autoantibodies in autoimmune disorders: crosstalk between Tfh/Tfr and regulatory B cells. Front Immunol 2021; 12: 641013.
[13] Huang X, Ran H, Li Y, Ma Q, Ou C, Qiu L, Feng H and Liu W. Leflunomide combined with low-dose prednisone inhibits proinflammatory T cells responses in myasthenia gravis patients. Front Neurol 2022; 13: 961628.
[14] Meng H, Zheng S, Zhou Q, Gao Y, Ni Y, Liang H and Chen S. FoxP3(-) Tr1 cell in generalized myasthenia gravis and its relationship with the Anti-AChR antibody and immunomodulatory cytokines. Front Neurol 2021; 12: 755356.
[15] Yang M, Long D, Hu L, Zhao Z, Li Q, Guo Y, He Z, Zhao M, Lu L, Li F, Long H, Wu H and Lu Q. AIM2 deficiency in B cells ameliorates systemic lupus erythematosus by regulating Blimp-1-Bcl-6 axis-mediated B-cell differentiation. Signal Transduct Target Ther 2021; 6: 341.
[16] Wang CC, Zhang M, Li H, Li XL, Yue LT, Zhang P, Liu RT, Chen H, Li YB and Duan RS. Caspase-1 inhibitor regulates humoral responses in experimental autoimmune myasthenia gravis via IL-6- dependent inhibiton of STAT3. Neurosci Lett 2017; 656: 169-176.
[17] Yu Q, Tu H, Yin X, Peng C, Dou C, Yang W, Wu W, Guan X, Li J, Yan H, Zang Y, Jiang H and Xia Q. Targeting glutamine metabolism ameliorates autoimmune hepatitis via inhibiting T cell activation and differentiation. Front Immunol 2022; 13: 880262.
[18] Wen Y, Yang B, Lu J, Zhang J, Yang H and Li J. Imbalance of circulating CD4(+)CXCR5(+) FOXP3(+) Tfr-like cells and CD4(+)CXCR5(+) FOXP3(-) Tfh-like cells in myasthenia gravis. Neurosci Lett 2016; 630: 176-182.
[19] Xie X, Mu L, Yao X, Li N, Sun B, Li Y, Zhan X, Wang X, Kang X, Wang J, Liu Y, Zhang Y, Wang G, Wang D, Liu X, Kong Q and Li H. ATRA alters humoral responses associated with amelioration of EAMG symptoms by balancing Tfh/Tfr helper cell profiles. Clin Immunol 2013; 148: 162-76.
[20] Baggi F, Annoni A, Ubiali F, Milani M, Longhi R, Scaioli W, Cornelio F, Mantegazza R and Antozzi C. Breakdown of tolerance to a self-peptide of acetylcholine receptor alpha-subunit induces experimental myasthenia gravis in rats. J Immunol 2004; 172: 2697-703.
[21] Sun C, Hao J, Qin H, Zhu Y, Li X, Zhang B, Qin Y, Li G, Wang H and Wang H. Endometrial regenerative cell-derived conditioned medium alleviates experimental colitis. Stem Cells Int 2022; 2022: 7842296.
[22] Sun J, Chen J, Xie Q, Sun M, Zhang W, Wang H, Liu N, Wang Q and Wang M. Sodium butyrate alleviates R97-116 peptide-induced myasthenia gravis in mice by improving the gut microbiota and modulating immune response. J Inflamm (Lond) 2023; 20: 37.
[23] Livak KJ and Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 2001; 25: 402-8.
[24] Shuey NH. Ocular myasthenia gravis: a review and practical guide for clinicians. Clin Exp Optom 2022; 105: 205-213.
[25] Salari N, Fatahi B, Bartina Y, Kazeminia M, Fatahian R, Mohammadi P, Shohaimi S and Mohammadi M. Global prevalence of myasthenia

gravis and the effectiveness of common drugs in its treatment: a systematic review and meta-analysis. J Transl Med 2021; 19: 516.
[26] Lehnerer S, Jacobi J, Schilling R, Grittner U, Marbin D, Gerischer L, Stascheit F, Krause M, Hoffmann S and Meisel A. Burden of disease in myasthenia gravis: taking the patient's perspective. J Neurol 2022; 269: 3050-3063.
[27] Nowak RJ, Coffey CS, Goldstein JM, Dimachkie MM, Benatar M, Kissel JT, Wolfe GI, Burns TM, Freimer ML, Nations S, Granit V, Smith AG, Richman DP, Ciafaloni E, Al-Lozi MT, Sams LA, Quan D, Ubogu E, Pearson B, Sharma A, Yankey JW, Uribe L, Shy M, Amato AA, Conwit R, O'Connor KC, Hafler DA, Cudkowicz ME and Barohn RJ; NeuroNEXT NN103 BeatMG Study Team. Phase 2 trial of rituximab in acetylcholine receptor antibody-positive generalized myasthenia gravis: the beatMG study. Neurology 2022; 98: e376-89.
[28] Kwon YN, Woodhall M, Sung JJ, Kim KK, Lim YM, Kim H, Kim JE, Baek SH, Kim BJ, Park JS, Seok HY, Kim DS, Kwon O, Park KH, Sohn E, Bae JS, Yoon BN, Kim NH, Ahn SW, Choi K, Oh J, Park HJ, Shin KJ, Lee S, Park J, Kim SH, Seok JI, Bae DW, An JY, Joo IS, Choi SJ, Nam TS, Kim S, Park KJ, Kwon KH, Waters P and Hong YH. Clinical pitfalls and serological diagnostics of MuSK myasthenia gravis. J Neurol 2023; 270: 1478-1486.
[29] McCombe JA and Pittock SJ. Anti-complement agents for autoimmune neurological disease. Neurotherapeutics 2022; 19: 711-728.
[30] Liu S, Chan J, Brinc D, Gandhi S, Izenberg A, Delgado D, Abdel-Qadir H, Wintersperger BJ and Thavendiranathan P. Immune checkpoint inhibitor-associated myocarditis with persistent troponin elevation despite abatacept and prolonged immunosuppression. JACC CardioOncol 2020; 2: 800-804.
[31] Malle L, Patel RS, Martin-Fernandez M, Stewart OJ, Philippot Q, Buta S, Richardson A, Barcessat V, Taft J, Bastard P, Samuels J, Mircher C, Rebillat AS, Maillebouis L, Vilaire-Meunier M, Tuballes K, Rosenberg BR, Trachtman R, Casanova JL, Notarangelo LD, Gnjatic S, Bush D and Bogunovic D. Autoimmunity in Down's syndrome via cytokines, CD4 T cells and CD11c(+) B cells. Nature 2023; 615: 305-314.
[32] Hong SB, Shin YW, Hong JB, Lee SK and Han B. Exploration of shared features of B cell receptor and T cell receptor repertoires reveals distinct clonotype clusters. Front Immunol 2022; 13: 1006136.
[33] Liang CL, Jiang H, Feng W, Liu H, Han L, Chen Y, Zhang Q, Zheng F, Lu CJ and Dai Z. Total glucosides of paeony ameliorate pristane-induced lupus nephritis by inducing PD-1 ligands(+) macrophages via activating IL-4/STAT6/PD-L2 signaling. Front Immunol 2021; 12: 683249.
[34] Oner F, Alvarez C, Yaghmoor W, Stephens D, Hasturk H, Firatli E and Kantarci A. Resolvin E1 regulates Th17 function and T cell activation. Front Immunol 2021; 12: 637983.
[35] Asashima H, Axisa PP, Pham THG, Longbrake EE, Ruff WE, Lele N, Cohen I, Raddassi K, Sumida TS and Hafler DA. Impaired TIGIT expression on B cells drives circulating follicular helper T cell expansion in multiple sclerosis. J Clin Invest 2022; 132: e156254.
[36] Gonzalez-Figueroa P, Roco JA, Papa I, Nunez Villacis L, Stanley M, Linterman MA, Dent A, Canete PF and Vinuesa CG. Follicular regulatory T cells produce neuritin to regulate B cells. Cell 2021; 184: 1775-1789, e19.
[37] Pan Z, Zhu T, Liu Y and Zhang N. Role of the CXCL13/CXCR5 axis in autoimmune diseases. Front Immunol 2022; 13: 850998.
[38] Hoehn KB, Turner JS, Miller FI, Jiang R, Pybus OG, Ellebedy AH and Kleinstein SH. Human B cell lineages associated with germinal centers following influenza vaccination are measurably evolving. Elife 2021; 10: e70873.
[39] Cui YZ, Qu SY, Chang LL, Zhao JR, Mu L, Sun B, Li HL, Zhang TS, Wang GY and Kong QF. Enhancement of T follicular helper cell-mediated humoral immunity reponses during development of experimental autoimmune myasthenia gravis. Neurosci Bull 2019; 35: 507-518.