Quercitrin ameliorates the development of systemic lupus erythematosus-like disease in a chronic graft-versus-host murine model

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Li W, Li H, Zhang M, Wang M, Zhong Y, Wu H, Yang Y, Morel L, Wei Q. Quercitrin ameliorates the development of systemic lupus erythematosus-like disease in a chronic graft-versus-host murine model. Am J Physiol Renal Physiol 311: F217–F226, 2016. First published February 24, 2016; doi:10.1152/ajprenal.00249.2015—Systemic lupus erythematosus (SLE) is a serious disorder of immune disease characterized by loss of immune tolerance and the production of autoantibodies, which result in the formation of immune complexes (6). Immune complex-mediated inflammation in SLE can lead to multisystem injury, including glomerulonephritis, arthritis, serositis, and blood dyscrasias (23). SLE is nine times more common in women of childbearing age than men, which indicates that women are more vulnerable to the disease. Among the SLE-like disease mouse models, chronic graft-versus-host disease (CGVHD) is a well-established model in which injection of parental DBA/2 spleen cells into (C57BL/6×DBA/2) F1 (BDF1) hybrids leads to a CGVHD response. The donor DBA/2 CD4+ T cells activate and expand host B cells with an autoreactive potential, leading to human SLE-like symptoms, such as proteinuria, autoantibody production, splenomegaly, and glomerulonephritis (16). Furthermore, female mice are more susceptible than males to CGVHD (13). During the development of the disease, CD4+ T cells play a critical role in regulating the autoimmune response. Common characteristics of the mouse strain combination used in CGVHD models involve disparities in class II major histocompatibility complex (MHC), indicating that stimulation of CD4 T cells is crucial in SLE-CGVHD (22). Chronic autoimmune diseases such as SLE develop through positive feedback from inflammation. Among the cells involved in the inflammation process, macrophages could participate via a number of mechanisms. CD4+ T cells that activate macrophages have a critical role in host defense against transplanted cells. After stimulation with some agents such as LPS, macrophages are involved in many autoimmune diseases through a number of mechanisms, and they play a critical role in these diseases. Recent studies have demonstrated that inflammation was also strongly associated in the CGVHD model. Inflammatory cytokines such as TNF-α were increased at both the mRNA and protein levels in macrophages and kidneys in a genetic SLE mouse model (25, 27). Taken together, stimulation of CD4+ T cells and inflammation mediated by the immune complex contribute to this CGVHD mouse model. The systematic screening of bacterial and fungal products has given us many important drugs, including cyclosporine A (CsA) and tacrolimus (FK506), which are now widely used to treat transplant recipients. CsA is a classic drug that blocks T cell proliferation by inhibiting the calcineurin pathway to modulate GVH responses (14). However, the use of CsA is often compromised by severe hepatotoxicity and nephrotoxicity (17). Consequently, there is a need for safer and better drugs, including natural compounds derived from human food or agriculture. Quercitrin (YDHH; Fig. 1A) is an abundant natural compound in Tartary buckwheat and swichgrasses (20, 29). Several studies have shown that YDHH has an anti-inflammatory effect, which is associated with an inhibition of inducible nitric oxide synthase (iNOS) and downregulation of the nuclear factor-κB pathway (11). However, little research has focused on the ability of YDHH to inhibit T cell activation. Our group has used YDHH as an immunosuppressant in different mouse models of immune disease, including delayed-type hypersensitivity and skin grafts. Based on the efficacy in these models (unpublished data), we hypothesized that YDHH can inhibit the stimulation of CD4+ T cells and exhibit an anti-inflam-
matory effect in the cGVHD mouse model. Thus the goal of this study was to test the effect of YDHH on the cGVHD-induced SLE-like disease in the mouse. A series of experiments was performed, including the in vivo effect of YDHH on proteinuria and serum autoantibodies, stimulation of CD4+ T cells and their cytokines, mRNA and protein expression of inflammatory cytokines, as well as the in vitro effect of YDHH on MAPK pathways.

MATERIALS AND METHODS

Reagents. Six- to eight-week-old female DBA/2 and (C57BL/6×DBA/2) BDF1 mice were purchased from Beijing Vital River. Mice were housed in a controlled environment (12:12-h light-dark period, 25°C) in the Animal Center of Beijing Normal University for 1 wk before the experiment. All experimental procedures were conducted according to protocols approved by the Ethic and Animal Welfare Committee (NO.CLS-EAW-2013-015) and were carried out in strict accordance with institutional guidelines.

A Bradford assay kit was purchased from Bioteke (Beijing, China). Blood urea nitrogen (BUN), triglyceride, and cholesterol test kits were bought from Reegen (Beijing, China). Calf thymus DNA, 3,3'–5,5'-tetramethylbenzidine (TMB), rabbit antibody against mouse IgG, goat anti-mouse IgG1 and IgG2, LPS (L2880), PMA, and ionomycin were purchased from Sigma (St. Louis, MO). Horseradish peroxidase (HRP)-conjugated rabbit anti-goat IgG and HRP-conjugated goat anti-mouse IgG were bought from CST (Danvers, MA). TRIzol, DMEM, and FBS were from Life Technologies (Carlsbad, CA). RNA and quantitative real-time PCR (qRT-PCR) reagents such as oligo/dT primers, TaqMan RT reagents, and SYBR Premix Ex Taq kit were from TaKaRa Bio (Kusatsu, Shiga, Japan). Flow cytometry antibodies such as FITC-conjugated anti-mouse CD4, PE-conjugated anti-CD8, anti-IFN-γ, and anti-IL-4 and Golgi Stop were bought from BD Biosciences (San Jose, CA). TNF-α, IL-1β, and IL-6 ELISA kits were from Neobioscience (Shenzhen, China). A nitric oxide (NO) kit was from Polygen (Beijing, China). Rabbit anti-mouse C3 antibody was from Abcam (Cambridge, UK). A CD4 separation kit and MACS separation columns were from Miltenyi. The BCA Protein Assay Kit was purchased from Life Technology and LPS was purchased from Sigma. YHHH was purified by Yu H. and Yang Y, and CsA was bought from Meilune (Dalian, China).

Murine model of cGVHD. Eight-week-old female DBA/2 mice were euthanized, and single-cell suspensions were prepared from the spleen. After being filtered through a sterile mesh, lysed in hemolysis buffer, and washed, 0.9% NaCl (control group) or 6×10⁷ DBA/2 spleen cells (other groups) per mouse were injected into the tail vein of BDF1 mice at day 7 and day 0. Mice were divided into five groups (n=7 each): control, untreated cGVHD, cGVHD treated with YDHH at a dose of 30 mg/kg or 80 mg/kg, and cGVHD treated with CsA (30 mg/kg). For additional mechanistic studies, mice were divided into three groups: control, cGVHD, and cGVHD treated with YDHH (80 mg/kg, n=4–6/group). YDHH and CsA were dissolved in 20% DMSO and 80% distilled water, respectively. YDHH and CsA were administered by daily gavage until the end of the experiment. Control and cGVHD groups received the same dose of vehicle (20% DMSO and 80% water, total of 0.2 ml·mouse·day⁻¹).

Determination of proteinuria, serum blood urea nitrogen, triglyceride, and cholesterol content. Serum and urine were collected every 2 wk. Mice were put in metabolic cages for urine collection. Proteinuria and albuminuria were tested with a Bradford assay and an ELISA kit. The results were determined with nanograms per day on the graph.
**Table 1. Primer sequences used for qPCR**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer 1 (Forward, 5’→3’)</th>
<th>Primer 2 (Reverse, 5’→3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-bet</td>
<td>ggctaacctggctggcagaagtgtt</td>
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<td>IL-6</td>
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</tr>
<tr>
<td>β-Actin</td>
<td>ggtcctgctctgggtaaggcagaa</td>
<td>cattggaatctggtttagaatg</td>
</tr>
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</table>

**RT-qPCR.** Total RNA was isolated from mouse tissues or cultured Cd4+ T cells using TRIzol (Life Technologies). Then, cDNA synthesis was initiated by adding oligo(dT) primers and TaqMan Reverse Transcription Reagents. qRT-PCR was performed according to the manufacturer’s instructions for the SYBR Premix Ex Taqkit (TaKaRa) with the Applied Biosystems 7500 fast Real-Time PCR system. β-Actin was used for normalization, and the sequences of the primers used are listed in Table 1.

**Flow cytometry.** Spleen cells were prepared as mentioned for the induction of the cGVHD part and processed into single-cell suspensions with an erythrocyte lysis solution and stimulated in vitro with 100 ng/ml PMA and 1,000 ng/ml ionomycin together with Golgi Stop for 4 h. Cells were then harvested, blocked, and stained with FITC-conjugated anti-mouse CD4 (GK1.5), PE-conjugated anti-mouse IFN-γ (XMG1.2), PE-conjugated anti-mouse IL-4 (11B11), and PE-conjugated anti-CD8 (Ly-2). Cells were analyzed on a FACSCalibur flow cytometer system with CellQuest software (BD).

**Western blotting.** Raw 264.7 cells were cultured in DMEM with 10% FBS. After having been treated with indicated concentrations of YDHH, CsA, and control solution, or 10 ng/ml LPS for 12 h, cells were lysed in cell lysis buffer, and protein concentrations were quantified using the BCA Protein Assay Kit. Western blotting was performed using primary antibodies against JNK, phospho-JNK, ERK, phospho-ERK, p38, phospho-p38, and β-actin and followed by ECL detection.

**Cytokine and NO measurement.** Cytokines in serum and culture supernatants, such as TNF-α, IL-4, IFN-γ, IL-1β, IL-10, and IL-6, were determined using either a Cytometric Bead Array kit (BD) or ELISA kit. Tests for NO in culture supernatants were performed using a NO test kit.

**Renal histopathology.** Mice were euthanized at week 10 for evaluating the histological scores in the kidneys. After having been fixed in 4% formalin and embedded in paraffin, kidneys were sectioned, mounted on slides, and stained with hematoxylin and eosin. Each observer was blinded when they evaluated each sec-
tion, scored the glomeruli, and classified the lesions (18). The following scale was applied: 0, normal morphology; 1, moderate expansion of the glomerular matrix without glomerulonephritis; 2, mild glomerulonephritis with mesangial hypercellularity and/or segmental necrosis; 3, moderate glomerulonephritis with extensive sclerosis and/or loop necrosis and/or cellular crescent; and 4, severe glomerulonephritis with large area of loop necrosis and cellular crescents.

**Immunofluorescence.** Kidneys were isolated from each group and immediately frozen into Tissue-Tek OCT compound. Sections (5-μm thickness) were cut on a cryostat, fixed in acetone, and washed successively in 1× PBS three times and blocked with 10% BSA in PBS for 30 min. Diluted rabbit anti-mouse C3 antibody (Abcam) was incubated overnight at 4°C, and an FITC-conjugated goat anti-rabbit IgG antibody (ZSGB-BIO) was used as a secondary antibody for C3. The fluorescence intensity of glomeruli from each animal was analyzed with ImageJ software. At least 15 glomeruli/section were analyzed.

**Statistical analyses.** The results are expressed as means ± SE. One-way ANOVA followed by Bonferroni’s Multiple Comparison tests were used to compare experimental groups and the control groups. The statistical analyses were performed with GraphPad Prism 5.0 software. The level of statistical significance was set at $P < 0.05$.

## RESULTS

**YDHH treatment decreased proteinuria and corrected serum triglyceride, BUN, and cholesterol in the cGVHD mouse model.** The presence of proteinuria is a critical indicator in the cGVHD model. First, mice were put in a metabolic cage for 24 h, and proteinuria among different groups of mice was monitored by an albumin ELISA or Bradford assay every 2 wk. As shown in Fig. 1B, all BDF1 mice in the cGVHD group exhibited albuminuria 10 wk after induction of cGVHD compared with the control group. YDHH and CsA treatments significantly lowered the amount of albumin in urine (Fig. 2). Proteinuria developed in the cGVHD group at week 6 and continued to increase in the following weeks. In contrast, both CsA and YDHH treatments significantly decreased the production of proteinuria (Fig. 1C). Triglycerides, BUN, and cholesterol also increased in the cGVHD mice from 6 to 10 wk after induction, and YDHH and CsA treatments lowered these serum indices, respectively (Fig. 1, D–F).

**YDHH treatment decreased serum antibodies in the cGVHD mouse model.** Anti-dsDNA IgG antibody is the signature antibody in SLE-like mouse models, as well as in human SLE (10). Serum anti-dsDNA IgG peaked at week 2 after the last cell injection and remained stable until week 10 in the cGVHD group. Serum anti-dsDNA IgG production was inhibited in mice treated with 30 and 80 mg/kg YDHH, as well as with CsA (Fig. 2A). Total serum IgG concentration was also significantly higher in the cGVHD group at week 10. However, it is inhibited in mice treated with 80 mg/kg YDHH (Fig. 2B).

Among serum IgG isotypes, IgG1 was elevated at week 10 after the second induction, while IgG2a was barely changed in the cGVHD group. YDHH significantly decreased IgG1 levels at 80 mg/kg, as well as CsA at 30 mg/kg (Fig. 2C). YDHH and CsA had little influence on IgG2a levels (Fig. 2D).

**YDHH alleviated renal pathology and SLE-like symptoms.** cGVHD induced immune complex-mediated nephritis. Renal histopathology was evaluated at 10 wk after cGVHD induction (Fig. 3A). Renal histopathology scores were significantly

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**Fig. 3.** YDHH ameliorated renal pathology and systemic lupus erythematosus (SLE)-like phenotypes in the cGVHD model 10 wk after induction. A: representative renal histopathology in each group (hematoxylin and eosin stain, ×20 and ×40). B: YDHH treatment attenuated histopathology score in BDF1 mice. C: representative spleen in each group and spleen weight. D: appearance of serum in each group. Values are means ± SE; $n = 5$. **$P < 0.01$ vs. control. # $P < 0.05$, ## $P < 0.01$ vs. cGVHD group.

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higher in the cGVHD group compared with the control group, and YDHH significantly lowered the scores (Fig. 3B). cGVHD mice showed splenomegaly compared with the control group, which was reduced by YDHH treatment (Fig. 3C). Furthermore, we found an aberrant amount of lacteal serum in the cGVHD mice model, indicating that the cGVHD mice were in serious metabolic disturbance (Fig. 3D). This result may reflect the aberrant serum biochemical index found in these mice, such as triglyceride, BUN, and cholesterol (Fig. 1, D–F). Contrary to YDHH, CsA did not improve either renal pathology or splenomegaly, which may be due to CsA hepatotoxicity and nephrotoxicity during the daily and long-term treatment.

**YDHH treatment alleviated CD4+ T cell activation in the cGVHD model.** CD4+ T cell activation and their interaction with CD8+ T cells, together with the presence of autoantibody-producing B cells, have been a major focus of investigation in SLE-cGVHD (3). Both CD4+ T cell expansion and ineffective CD8+ T cells play a role in this model (6). The frequency of splenic CD4+ T cells as well as the CD4/CD8 T cell ratio were elevated in the cGVHD group, and these phenotypes were
reduced by YDHH treatment (Fig. 4, A and B). This suggested that YDHH might ameliorate SLE via inhibition of CD4+ T cells. It is also known that stimulation of CD4 T cells is crucial in SLE-cGVHD (22). Both IFN-γ and IL-4 production were increased after in vitro stimulation with PMA and ionomycin of CD4+ T cells in the cGVHD group. The YDHH treatment markedly decreased the secretion of these cytokines (Fig. 4, C–E).

It is well known that CD4 Th1/Th2 differentiation plays an essential role in cGVHD mouse model. As SLE is a multiorgan disease, we looked into the key transcription factors Th1 (T-bet) and Th2 (GATA-3) in different tissues at week 8. We found that only the spleen of cGVHD mice exhibited a very high expression of T-bet and GATA-3, while there was no difference in the liver, lung, or kidney (Fig. 5A). To determine the effect of YDHH on spleen CD4+ T cells, purified spleen CD4 T cells were stimulated with PMA and ionomycin for 4 h, then total RNA was isolated and prepared for qRT-PCR, and culture supernatants were harvested for ELISA. Both T-bet (5-fold) and GATA-3 (1.5-fold) were increased in the cGVHD group compared with the control group. YDHH treatment significantly decreased the expression of these transcription factors (Fig. 5B). In addition, YDHH reduced the secretion of IL-2, IFN-γ, and IL-4 compared with the cGVHD group, while IL-10 was not affected (Fig. 5C).

YDHH showed anti-inflammatory effects in both kidney and peritoneal macrophages in the cGVHD model. At week 8, peritoneal macrophages were harvested and cultured with or without LPS to test the cytokines they produced by ELISA. Figure 6A shows that the inflammatory cytokines TNF-α and IL-1β were secreted in peritoneal macrophages with or without LPS. Both of these inflammatory factors were increased in the cGVHD group, regardless of whether they were induced by LPS. YDHH treatment markedly decreased the secretion of these two factors when treated with LPS.

Many studies have focused on the inflammatory factors that are overactivated in the kidney in SLE (24, 27). We used qRT-PCR to determine the inflammatory factors in the kidney in our model. The mRNA expression of TNF-α and IL-6 was decreased in the YDHH group compared with the cGVHD group (Fig. 6B). Finally, IgG immune complex deposition (C3) was exacerbated greatly in the cGVHD model while YDHH reduced it (Fig. 6C). Taken together, these suggested that the anti-inflammatory effects of YDHH might protect against the disease.

YDHH manifested anti-inflammatory effect in the Raw264.7 cell line. Next, we analyzed the anti-inflammatory effect of YDHH in vitro in the RAW264.7 cell line. First, the effect of YDHH on its activity in an MTT assay was used to test the toxicity of YDHH in Raw264.7 cells. YDHH did not exhibit cell toxicity in the range of 100–800 μM with or without LPS for 24 h (data not shown). Raw264.7 cells were treated with vehicle (control, white bar), LPS (positive control, black bar), CsA, or YDHH for 12 h. LPS was then added for another 24 h, and the supernatants were collected for an ELISA. After pretreatment with different concentrations of YDHH, TNF-α, IL-6, and NO secretions were significantly decreased in a dose-dependent manner compared with the LPS-positive control (Fig. 7, A–C). To confirm whether the inhibition of NF-κB activation is mediated through the MAPK pathways, we tested...
the effect of YDHH on LPS-stimulated phosphorylation of ERK1/2, JNK, and p38 MAPK in RAW264.7 cells. As shown in Fig. 7D, LPS notably induced phosphorylation of ERK1/2, JNK, and p38. Pretreatment with YDHH significantly inhibited the LPS-stimulated phosphorylation of p38 (0.2 and 0.5 mM), JNK (0.2 and 0.5 mM), and ERK (0.1, 0.2 and 0.5 mM) (Fig. 7E). This finding suggests that YDHH inhibited phosphorylation from all three MAPK pathways, with a maximum inhibitory effect on ERK1/2.

DISCUSSION

There are many murine models of SLE, including the New Zealand strains and the MRL/lpr mice, in which genetic variation drives the loss of immune tolerance (7, 9). The chronic GVHD mouse model is a well-established murine model which induces SLE by injecting DBA/2 spleen cells into BDF1 mice. The cGVHD model is easier to control, more adjustable, and has a shorter period compared with the genetic models. In addition, this parent to the F1 mouse model resembles SLE because it is a sex-bias GVHD model, in which the female donor will make the female host more vulnerable to SLE than a male (8, 13). All these reasons make cGVHD an ideal model with which to study SLE. This article examines the impact of quercitrin on the development of a SLE-like disease in a cGVHD mouse model. We found that daily administration of 80 mg/kg of quercitrin ameliorated the SLE symptoms in this model. In the present study, our results indicate that quercitrin treatment strongly prevented the development of proteinuria (Fig. 1, B and C), corrected the aberrant biochemical index in serum (Fig. 1, D–F), decreased serum antibody production (Fig. 2), and consequently ameliorated the SLE-like nephritis symptoms (Fig. 3). YDHH also inhibited the activation of CD4 T cells (Figs. 4 and 5) and macrophages (Fig. 6). Finally, the anti-inflammatory effect of quercitrin was confirmed in the Raw264.7 cell line (Fig. 7). The therapeutic effect of quercitrin may, at least partially, contribute to the inhibition of CD4 T cells and its anti-inflammatory effect.

It has traditionally been assumed that the predominant cytokines produced in acute GVHD are Th1 cytokines, while those produced in chronic GVHD are Th2 cytokines. Th1 cells prefer-
entially produce IL-2 and IFN-γ, activate macrophages, and stimulate the production of IgG2a (12, 21). Th2 cells produce cytokines such as IL-4 and IL-10 and induce the production of IgG1 in mice (4). However, recent studies suggest that cGVHD could be caused by cytokines secreted by Th1 cells or autoantibodies (15, 26, 28). In our present study, serum IgG1 (Fig. 2C) and IL-4 were increased in the cGVHD mouse model, indicating that there is a skewing toward Th2 cells. However, we found both spleen Th1 cells and Th2 cells were activated after stimulation in vitro by PMA and ionomycin at week 8. We chose the characteristic Th1 cytokine IFN-γ and Th2 cytokine IL-4 for intracellular staining and found both IFN-γ and IL-4 were increased in cGVHD mice (Fig. 4, D and E). Meanwhile, the mRNA expression of T-bet and GATA-3, key transcription factors of Th1 and Th2 cells, were tested directly from the spleen, lung, kidney, and liver, and we found there was significantly higher expression of T-bet and GATA-3, as well as IFN-γ, in the spleen but not in other tissues (Fig. 5A). In addition, both Th1 and Th2 CD4+ T cells were expanded after PMA/Ionomycin stimulation (Fig. 5, B and C). At week 10, we found splenomegaly in cGVHD mice due to the GVH reaction (Fig. 3). Necrosis in spleen cells could activate macrophages through IFN-γ, then induce the Th1 expansion. It may contribute to the expansion of Th1 cells in our present study.

Fig. 8. Potential mechanism of action of YDHH in cGVHD mouse model: cGVHD in DBA/2 to BDF1 (C57BL/6×DBA/2) mice. Donor CD8-mediated, anti-recipient MHC I is defective, while CD4 T cells are stimulated and help B cells produce an antibody. YDHH lowers the secretion of IFN, IL-2, and IL-4, then alleviates the activation of CD4 T cells and further decreases the autoantibody. YDHH also shows inflammatory effects on both the kidney and macrophages that might further decrease the proteinuria and ameliorate the SLE disease.
It has been reported that quercitrin suppressed LPS-mediated NF-κB activation and downregulated LPS-induced NO production and iNOS expression (11). Quercitrin can also inhibit AP-1 and MAPK signaling in J85 p+ cells (5), indicating that its inflammatory effect through the MAPK signaling pathway may play an important role in the nephritis model. We used ELISA and qRT-PCR experiments to measure the anti-inflammatory effect of YDHH in both kidney and peritoneal macrophages in the cGVHD model. The YDHH group exhibited a lower cytokine expression (Fig. 6). During our experiment, we found that YDHH attenuated splenic T cell activation and reduced renal cytokine levels. However, we were unable to successfully stain the kidney tissues to evaluate T cell and macrophage infiltration (data not shown). Thus it remains unknown whether YDHH attenuated T cell or macrophage infiltration in the kidney. Furthermore, we used the RAW264.7 cell line to discover that quercitrin suppressed LPS-mediated inflammation through the MAPK pathway (Fig. 7). In our experiment, YDHH showed no toxic effect with Raw264.7 cells with a 100–800 μM range (data not shown), but YDHH required a higher concentration (0.1–0.5 mmol) to exhibit its significantly anti-inflammatory effect compared with CsA (0.01 mmol) in RAW264.7 cells.

The immunosuppressive drug CsA is believed to inhibit primarily the activation of T helper and B lymphocytes by interfering with the synthesis and release of IL-2 (19). It has been reported that early CsA treatment with 250 mg·kg⁻¹·wk⁻¹ can prevent glomerulosclerosis in this lupus nephritis model (1). Meanwhile, CsA was also found to markedly potentiate both the splenomegaly and hyper-IgE response at low doses before exhibiting an inhibitory effect at higher doses (2). In our present study, daily oral gavage of 30 mg/kg CsA lowered the proteinuria and corrected the aberrant biochemical index in serum. CsA also decreased serum antibodies. However, a significant body weight change in the CsA-treated mice and abdominal edema were observed during the experiment (data not shown). Due to the edema, the CsA-treated mice also exhibited less physical activity than other groups. It is speculated that oral gavage CsA might lead to long-term reaction, then the abdominal edema. This might link to its toxicity. Furthermore, the renal histopathology of the CsA group showed moderate glomerulonephritis with extensive sclerosis at week 10. This result may link with the observed nephrotoxicity because of the long-term oral CsA treatment. In conclusion, quercitrin treatment possesses similar effects but less toxicity compared with CsA.

In a nutshell, the potential mechanism of action of YDHH might be the inhibition of CD4+ T cells and the anti-inflammatory effect against macrophages. As we can see in Fig. 8, cGVHD was induced by injecting DBA2 spleen cells into BDF1 mice. Donor CD4+ T cells are stimulated and help B cells produce antibody while CD8+ T cells are defective. After CD4+ T cell activation, the elevated secretion of cytokines such as IFN-γ, IL-2, and IL-4 helps B cells to produce antibodies. These overproductive antibodies assemble in the kidney, then form the deposition of immune complexes. The inflammation effect caused by immune complexes finally results in renal failure and proteinuria. In our present study, YDHH inhibited CD4+ T cells as well as the cytokines they produce and then lowered the production of antibodies such as anti-dsDNA IgG. Meanwhile, YDHH also showed an anti-inflammatory effect against macrophages both in the mouse model and in the Raw cell line and finally ameliorated the SLE-like disease in the cGVHD model.

In conclusion, quercitrin is a natural compound that can be isolated from buckwheat. The experimental data in this study showed that it is effective as an immunosuppressive agent, and the therapeutic effect was associated with the downregulation of both stimulation of T cells and inflammation.

GRANTS
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
W.L. and Q.W. provided conception and design of research; W.L., H.L., M.Z., M.W., Y.Z., H.W., and Y.Y. performed experiments; W.L., and H.L. interpreted results of experiments; W.L., and H.L. prepared figures; W.L. and Q.W. drafted manuscript; W.L., H.L., M.Z., M.W., Y.Z., H.W., Y.Y., L.M., and Q.W. edited final version of manuscript; L.M. and Q.W. edited and revised manuscript.

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