

# Longdan Xiegan Tang has immunomodulatory effects on CD4<sup>+</sup>CD25<sup>+</sup> T cells and attenuates pathological signs in MRL/*lpr* mice

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**Abstract.** *Longdan Xiegan Tang* (LXT) is a Chinese herbal medicine that is prescribed as an anti-inflammatory aid, a hepato-protectant, and an immunostimulant. In this study, we examined the biological effects of LXT administration in MRL/*lpr* mice. MRL/*lpr* mice provide a good model of systemic lupus erythematosus (SLE). These mice develop immunological disturbances and dysregulation in Th1 and Th2 cytokine production. Female mice were randomly separated into two groups. The experimental group received LXT (250 mg/kg/day, po) from 19 to 21 weeks of age. Splenic CD3<sup>+</sup>CD4<sup>+</sup>, CD3<sup>+</sup>CD8<sup>+</sup>, and CD4<sup>+</sup>CD25<sup>+</sup> T cells were increased in the LXT-administered mice compared to MRL/*lpr* controls, and this was associated with splenomegaly. There was a marked reduction in IFN- $\gamma$ , TNF- $\alpha$ , anti-dsDNA antibody, and there were reduced IgG immune complex deposits in the glomeruli. LXT also restored kidney glutathione levels, thereby limiting the toxic effects of the inflammatory mediators iNOS and COX-2, which are overproduced in MRL/*lpr* mice. Two-dimensional gel electrophoresis was used to analyze proteome changes. LXT protected MRL/*lpr* mice against developing the lupus syndrome through up-regulation of phosphoglycerate kinase 1 and down-regulation of ferritin light chain 1, selenium-binding protein 2, and  $\alpha$ -enolase. This study indicates that LXT at this dose and time course of administration was effective in reducing oxidative stress associated with disease progression in MRL/*lpr* mice. LXT could be useful as adjunctive therapy for reducing distress in SLE.

## Introduction

The pathogenesis of systemic lupus erythematosus (SLE) is multifactorial and polygenic. The apoptosis gene *Fas* is a

candidate contributory gene in both human SLE and murine models of SLE. The MRL/*lpr* mouse is a prototypical model for human SLE in which the presence of a single gene mutation on the *Fas* (CD95) gene leads to reduced signaling for apoptosis (1). The impaired clearance of apoptotic cells favors self-antigen presentation and the production of multiple autoantibodies, which represents the central immunological disturbance in murine SLE (2). The presence of anti-double-stranded DNA (anti-dsDNA) IgG and anti-tissue-transglutaminase (anti-tTG) IgG (3), splenomegaly, and deregulated production of Th1 and Th2 cytokines (4) have been used as markers to monitor murine SLE disease progression. However, *lpr* mutation alone cannot account for the entire autoimmune syndrome of MRL/*lpr* mice. Other genes of pathologic importance in the MRL background have also been mapped (5,6).

The dysregulation of immune T-cell tolerance is a crucial event in the production of pathogenic autoantibodies and the general pathogenesis of both human and murine SLE. T cells have an important role in the control of autoimmunity, and are low in number and/or function in lupus-prone mice and in SLE patients with active disease (7,8). Oxidative stress has also been implicated in the pathogenesis of several degenerative diseases, such as Alzheimer disease, Parkinson disease, systemic lupus erythematosus, and cancer. In particular, its involvement in autoimmune disease has been supported by the increase of oxidative stress markers (9,10) and by the preventive effect played by the dietary intake of antioxidants (11).

In clinical application, most traditional Chinese medicines (TCMs) have been prescribed as a combination of more than two herbal crude drugs to obtain any additive effects and to diminish the possible adverse responses. The traditional Chinese prescription *Longdan Xiegan Tang* (LXT, Chinese name) is composed of ten important plants: *Gentiana rigescens* Franch, *Scutellaria baicalensis* Georgi, *Gardenia jasminoides* Ellis, *Alisma orientalis* (Sam.) Juzep, *Clematis Montana* Buch.-Ham., *Plantago asiatica* L, *Angelica sinensis* (Oliv.) Diels, *Rehmannia glutinosa* Libosch, *Bupleurum chinense* DC, and *Glycyrrhizae uralensis* Fisch. LXT is one of the most-prescribed Chinese herbal formulas for patients with chronic hepatitis in Taiwan (12). It is also widely used in Chinese medication for its anti-inflammatory, anti-infection, anti-bacterial, anti-allergy, hepatoprotectant, cholagogic, and immunostimulatory properties (13).

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The first set of experiments in this study addressed the question of whether LXT administration can modulate splenomegaly, cytokine production, autoantibody synthesis, and redox status in MRL/*lpr* mice. Next, the beneficial effects of LXT on typical kidney pathological signs of MRL/*lpr* mice were investigated, and the involvement of biochemical mechanisms was examined. Significant amelioration of typical pathological signs in MRL/*lpr* mice by short-term LXT administration was accompanied by T cells modulation and improved cytoprotective defenses in kidney. This was accompanied by decreased inflammation and oxidative stress signaling.

## Materials and methods

**Mice and LXT treatment.** Six-week-old female MRL/*lpr* mutant mice were originally purchased from Jackson Laboratory (Bar Harbor, ME). Balb/C mice were purchased from the Animal Center of Taiwan University (Taipei). The mice were housed in specific pathogen-free conditions in our animal facilities. All animal protocols were approved by the Institutional Animal Care and Use Committee at the Chang Gung University. Twenty mice were randomly separated into 4 cages, with 5 mice per cage. Two cages were in each study group for a total of 10 mice per study group. At 19 weeks of age, the mice received LXT for 2 weeks. LXT decoctions were boiled together with 350 ml water for 1 h and filtered. This was repeated three times, and the filtrates were combined and concentrated to 200 ml by rotary evaporation at 80°C. LXT was then dissolved in distilled water and administered daily (250 mg/kg, po) over the experimental period. Control mice received equal volumes of distilled water. Medicinal plants (crude drugs) used for preparation of LXT were purchased from Chuang Song Zong Pharmaceutical Co., Ltd. (Pingtung, Taiwan). All of these plant materials were authenticated by Dr R.-J. Yang and voucher specimens were deposited in Chang Gung Memorial Hospital, Center for Chinese medicine pharmacy, Taoyuan, Taiwan.

**Phenotyping and flow cytometric analysis.** At 21 weeks of age, mice were weighed, sacrificed and necropsied. The spleens were removed, weighed, aseptically dissociated, and treated with Tris-ammonium chloride-lysis buffer (pH 7.2) to remove erythrocytes. Flow cytometric analysis was performed using fluorescein-isothiocyanate (FITC)-conjugated CD4 and CD8 anti-mouse monoclonal antibodies, and phycoerythrin (PE)-conjugated CD11b, CD3 and CD25 anti-mouse monoclonal antibodies (BD Pharmingen, San Diego, CA). PBS (100  $\mu$ l) with the relevant monoclonal antibody diluted to the equivalent of 1  $\mu$ g/ $1 \times 10^6$  cells was added to the cells. The cells were incubated for 30 min at 4°C. After 30 min, cells were washed with 0.1% BSA in PBS, and analyzed by flow cytometry using a FACSCalibur flow cytometer (BD Biosciences). Data were analyzed using the CellQuest software program (Becton-Dickinson, USA). For each sample, 10,000 events were collected.

**Histopathology and immunohistochemistry.** At the time of necropsy, the kidneys were removed and divided into sections. One portion was placed in buffered formalin and subsequently

embedded in paraffin, sectioned, and stained with H&E and IgG. Sections were assessed via light microscopy for glomerular proliferation, glomerular inflammation and glomerular size. Glomeruli were detected by incubating with horseradish peroxidase-conjugated goat anti-mouse IgG (1:200, Vector Laboratories, Burlingame, CA). Sections were then counterstained with hemalum. The pathology and morphometric analysis were performed by a pathologist blinded to the groups (Dr Y.-C. Chu).

**Anti-dsDNA autoantibody, cytokine, and glutathione detection.** Anti-dsDNA autoantibody levels were quantified by ELISA. dsDNA was isolated by S1 nuclease (Sigma-Aldrich) treatment of phenol-extracted calf thymus DNA. Ninety-six-well ELISA plates were then coated with 5  $\mu$ g/ml calf thymus dsDNA, and incubated at 37°C overnight. The plates were washed with PBS containing 0.05% Tween (PBS-T). Serum was added to each well in serial dilutions starting at a 1/100 dilution, and incubated for 45 min at room temperature. After washing with PBS-T, HRP-conjugated goat anti-mouse IgG ( $\gamma$ -chain specific; Sigma-Aldrich) was added and incubated for 45 min. After thorough washing, 0.1 M citrate buffer (pH 4.0) containing 0.015% H<sub>2</sub>O<sub>2</sub> and 3,3',5,5'-tetramethylbenzidine (Sigma-Aldrich) was added. The results are reported as the absorbance at 380 nm at a 1/100 serum dilution. Serum levels of IFN- $\gamma$ , TNF- $\alpha$ , IL-4 and IL-5 in the supernatants were quantified by ELISA, following the manufacturers' instructions (R&D Systems). Changes in kidney GSH and GSSG were determined by HPLC (14).

**Western blotting and 2-D gel electrophoresis.** Kidney tissue was lysed with distilled water containing proteinase inhibitors (BD Pharmingen), and then sonicated. After using a Bio-Rad rapid Coomassie kit to determine total protein concentration, 60  $\mu$ g of protein was subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Western blotting. The proteins were transferred onto a polyvinylidene difluoride membrane, blocked with 5% milk solids, and exposed to various mouse or rabbit monoclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) or anti- $\beta$  actin antibody (Sigma). Chemiluminescence (ECL; Amersham, Piscataway, NJ) in conjunction with video densitometry was used to quantify protein expression. Two-dimensional-PAGE was performed according to our previous study to detect various proteosomal changes (15).

**Statistical analysis.** The results are expressed as means  $\pm$  SEM. Statistical analysis were performed using one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls multiple range test. Statistical difference was accepted at  $P < 0.05$ .

## Results

**LXT reduces skin lesions and splenomegaly in MRL/*lpr* mice.** Lupus affects the skin, causing rashes and lesions, usually on the face and upper torso. The cutaneous lesions of MRL/*lpr* mice resemble those that appear in human SLE. MRL/*lpr* mice develop necrotic skin lesions on their ears, hair loss, and scab formation, typically on the upper back (Fig. 1A). In LXT

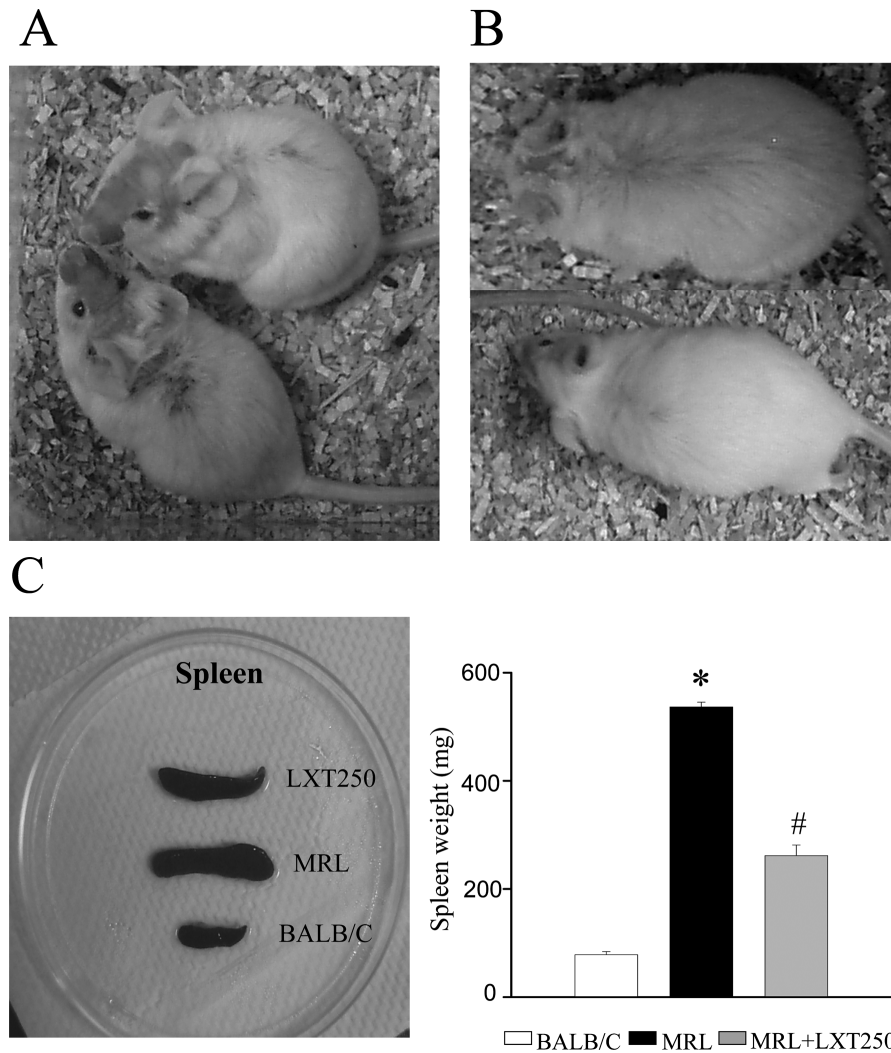


Figure 1. Regression of skin lesions in LXT-treated MRL/lpr mice. (A) MRL/lpr mice display the characteristic signs of skin lesions on the back and ear typically exhibited at 19 weeks. (B) Mice treated with LXT (250 mg/kg) daily for 2 weeks show no signs of the skin involvement or ear necrosis that is typically seen in MRL/lpr mice at 21 weeks of age. (C) Spleen from Balb/C mice, MRL/lpr mice, and MRL/lpr mice administered LXT. The abnormally high spleen weights were reduced under LXT treatment.

treated animals, skin lesions were dramatically attenuated. Administration of LXT (250 mg/kg/d, for 2 weeks) to MRL/lpr mice led to both the quasi-total disappearance of cervical cutaneous lesions and hair regrowth (Fig. 1B). At 21 weeks of age, the mice were sacrificed, and the splenic weights were determined. The weights of spleens of LXT-treated mice were significantly decreased compared with the untreated mice (Fig. 1C).

**Expression of CD11b, CD4<sup>+</sup>, CD8<sup>+</sup> and CD4<sup>+</sup>CD25<sup>+</sup>-positive spleen cells.** To further investigate the splenic profiles in the LXT-treated animals, we analyzed the splenic composition of cells by flow cytometry. The flow cytometry results showed that both CD11b (Fig. 2A) and two different T cell populations, CD3<sup>+</sup>CD4<sup>+</sup> and CD3<sup>+</sup>CD8<sup>+</sup>, were decreased in the MRL/lpr mice (Fig. 2B and C, respectively). The LXT-treated animals had a significant increase in the number of T cells. Interestingly, percentages of CD4<sup>+</sup>CD25<sup>+</sup> T cells were slightly (but statistically significantly) higher in the LXT group compared to the untreated MRL/lpr mice (Fig. 3). The percentages of these two groups were also lower when compared to the

Balb/C group. LXT-treated animals showed a significant increase in the number of Treg cells both in blood and spleen. These results indicate that LXT may modulate Treg cell activation.

**Modulation of Th1/Th2 cytokines in MRL/lpr mice following LXT administration.** IFN- $\gamma$ , TNF- $\alpha$ , IL-4, and IL-5 are major mediators of several autoimmune and inflammatory diseases. We thus compared their concentrations in the serum of MRL/lpr and MRL/lpr mice treated with LXT. As expected, MRL/lpr mice had much higher levels of TNF- $\alpha$  (Fig. 4A) and IFN- $\gamma$  (Fig. 4B), and had lower IL-4 (Fig. 4C), and IL-5 (Fig. 4D) levels, while LXT treatment restored normal cytokine concentrations in MRL/lpr mice.

**LXT effects on autoantibody production.** Importantly, the reduction of serum Th1 cytokines paralleled the decrease in anti-dsDNA levels in MRL/lpr mice treated with LXT (250 mg/kg). As MRL/lpr mice age, they exhibit high concentrations of autoantibodies directed against several nuclear and extracellular autoantigens, including dsDNA. To determine

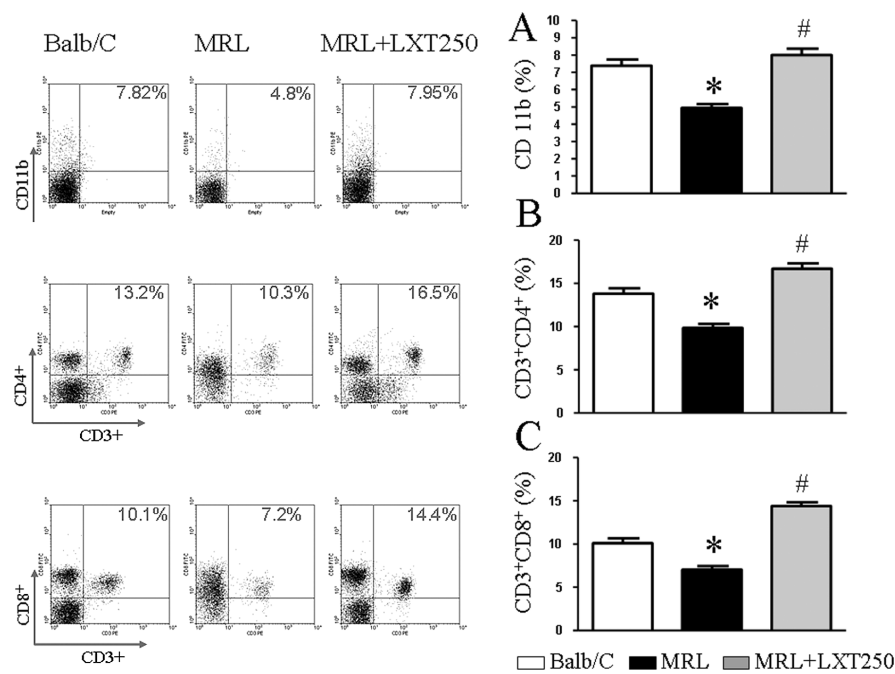


Figure 2. Flow cytometric analysis of splenic T cell and CD11b cell activation markers in LXT-treated or untreated MRL/*lpr* mice. (A) Splenic CD11b cells. (B) Representative dot plot data showing the percentage of cells that stained positive for CD3+CD4+ or CD3+CD8+ in MRL/*lpr* mice and in 250 mg/kg LXT-treated animals. The graph shows that CD3+CD4+ and CD3+CD8+ T cells were significantly increased in both the Balb/C and LXT-treated groups compared with MRL/*lpr*. Splenic T cells were analyzed for different T cell surface markers. Data represents the mean  $\pm$  SEM of at least six mice at 21 week of age (\*compared with Balb/C, #compared with MRL/*lpr*,  $p < 0.05$ ).

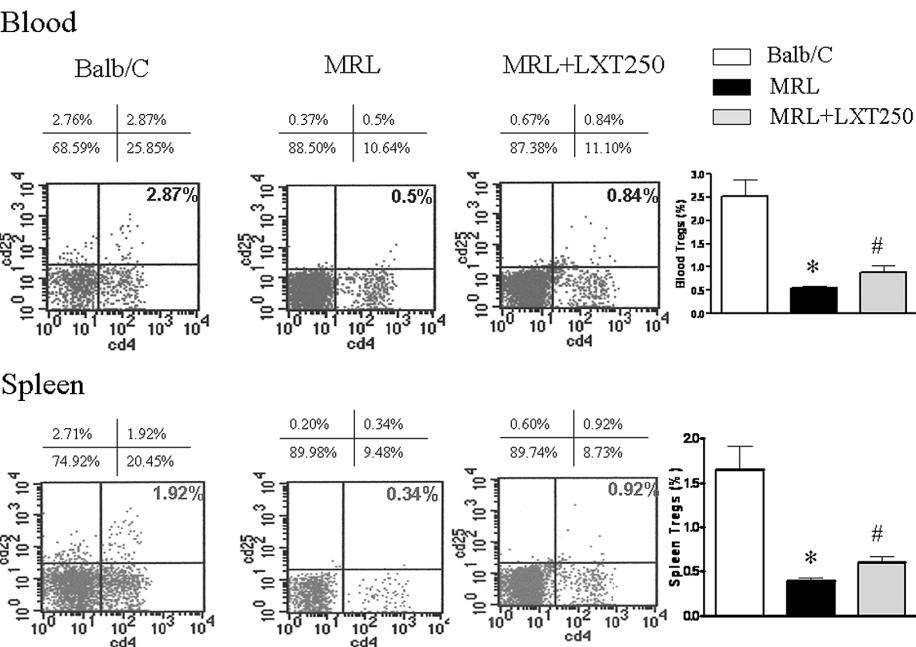


Figure 3. Flow cytometric analysis both of blood and splenic CD4+CD25+ T cell activation markers in LXT treated or untreated MRL/*lpr* mice. The representative dot plot data show the percentage of cells that stained positive for CD4+CD25+ in Balb/C, MRL/*lpr*, and 250 mg/kg LXT treated mice. The graph shows that CD4+CD25+ T cells were significantly increased in both Balb/C and LXT-treated groups compared with MRL/*lpr* (\*compared with Balb/C, #compared with MRL/*lpr*,  $p < 0.05$ ). Data represent the mean  $\pm$  SEM of at least six mice at 21 weeks of age.

whether treatment with LXT modified autoantibody production, serum levels of anti-dsDNA autoantibodies were quantified. Compared with MRL/*lpr* mice, the levels of anti-dsDNA autoantibodies in LXT-treated mice were significantly decreased in this experiment (Fig. 4E).

**Renal pathology.** To assess the effects of LXT on kidney function, kidney sections were stained with H&E and for IgG and were scored by a pathologist that was blinded to the treatment groups (Fig. 5). MRL/*lpr* mice showed typical glomerulonephritis, characterized by enlarged glomeruli, proliferation



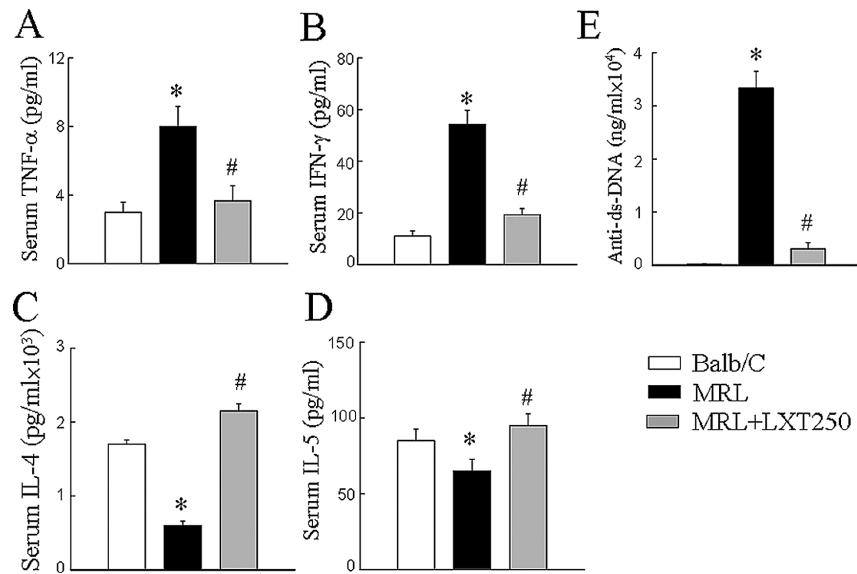


Figure 4. Effects of LXT treatment on cytokine and autoantibody production in MRL/lpr mice. Serum was harvested from these mice and assayed for the cytokines TNF- $\alpha$  (A), IFN- $\gamma$  (B), IL-4 (C), IL-5 (D), and anti-dsDNA autoantibody (E) levels by ELISA. Results from six to eight mice in each group are expressed as mean  $\pm$  SEM (\*P<0.05 compared with the Balb/C group, #P<0.05 compared with the MRL/lpr group.)

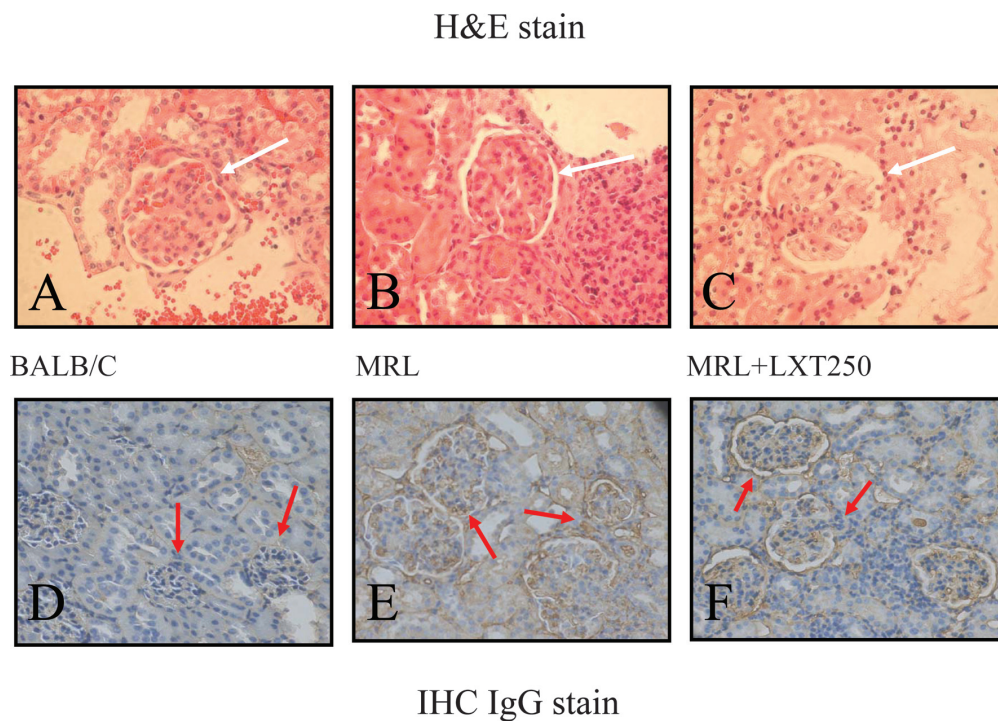


Figure 5. Representative photomicrographs show a pathologic glomerulus from Balb/C, MRL/lpr untreated, and MRL/lpr mice treated with LXT (250 mg/kg BW) for 2 weeks beginning at 19 weeks of age. At the time of sacrifice (21 weeks), the kidneys were removed, then sectioned before staining with H&E (A, B and C), or for IgG (D, E and F).

of glomerular cells, infiltration of inflammatory cells, and increased mesangial matrix (Fig. 5B). In contrast, mice treated with LXT showed a lesser degree of glomerulonephritis, varying degrees of focal glomerular cell proliferation, occasional inflammatory cells, and only a slight increase in the mesangial matrix (Fig. 5C). These results indicate that LXT administration inhibited proliferative renal disease in MRL/lpr mice. Treatment of MRL/lpr mice with LXT for

2 weeks significantly reduced glomerular IgG deposits compare with MRL/lpr mice (Fig. 5E vs. 5F). Additionally, LXT-treated mice displayed no thickening in glomerular basement membranes in histological evaluation.

*GSH expression is associated with inhibition of inflammatory mediator in the kidney of MRL/lpr mice.* As an inefficient antioxidant defense system has been linked to the progression

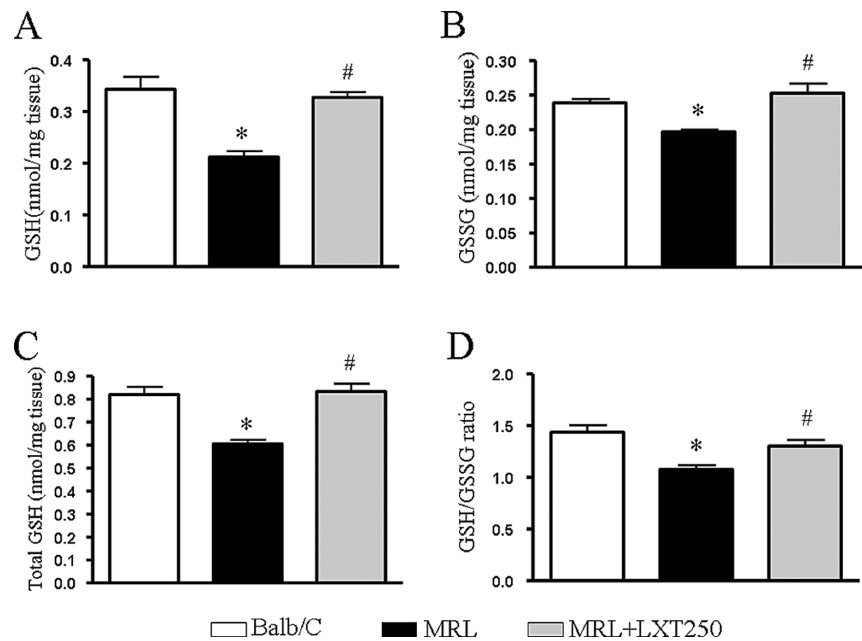


Figure 6. Reduced glutathione (GSH) (A), oxidized (GSSG) glutathione (B), total glutathione (GSH+GSSG) (C), and GSH/GSSG ratio (D) determined in kidneys from Balb/C, MRL/*lpr*, MRL/*lpr* mice treated with LXT (250 mg/kg BW). Each bar represents the mean value of experiments performed in triplicate assays  $\pm$  SEM (n=8). (\*P<0.05 compared with the Balb/C, #P<0.05 compared with the MRL/*lpr* group.)

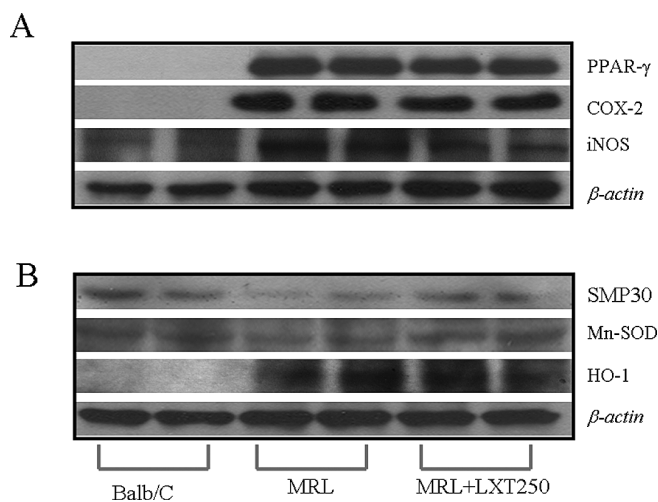


Figure 7. Western blot analysis of kidney tissue protein. Kidney homogenate fractions (60  $\mu$ g protein per lane) were analyzed for immunoreactivity with specific antibodies, as described in Material and methods section.  $\beta$ -actin was used as an internal control.

of autoimmune diseases, the alteration of GSH concentration was evaluated. As expected, a significantly lower GSH concentration was found in kidney tissue from MRL/*lpr* mice compared to normal control mice (Fig. 6). LXT treatment leads to much higher concentrations of total GSH in the kidney than are found in MRL/*lpr* mice. Notably, the significant increase in GSH concentration was associated with an increase in oxidative stress marker proteins SMP30, Mn-SOD and HO-1 levels as shown by Western blot (Fig. 7B). Finally, the inhibition of oxidative stress exhibited by LXT has been associated with PPAR- $\gamma$  inhibition and the subsequent blockade

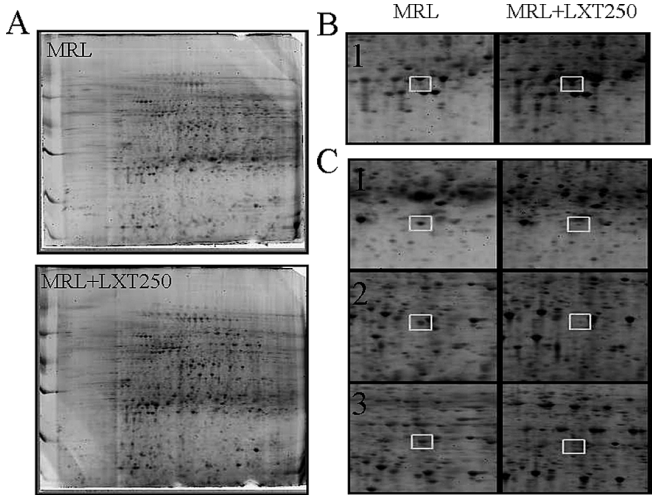


Figure 8. Partial 2D images of kidney tissue proteins from MRL/*lpr* mice and mice receiving LXT treatment (A). The protein samples were analyzed as stated under the Materials and methods. Examples of spots representing proteins whose level changed due to the LXT treatment are highlighted (B and C), and the measured protein levels for phosphoglycerate kinase 1 is shown (B1); ferritin light chain 1, selenium-binding protein 2, and  $\alpha$ -enolase are also shown (C1-3).

of inflammatory protein expression (e.g., iNOS and COX-2) (Fig. 7A).

**Proteomics analysis in kidney tissue of MRL/*lpr* mice by LXT.** A proteomic analysis was conducted to enhance our understanding of the fundamental aspects of how LXT exerts its protective effect on MRL/*lpr* mice, and to provide potential insights that could impact clinical practice. To our knowledge, this is the first description of kidney proteome alterations

Table I. Summary of expression data and identified proteins from the kidney of MRL/*lpr* mice after *Longdan Xiegan Tang* treatment.

No.	Accession no.	Protein name (fold-changes)	MRL vs. Exp	Function
1	P09411	Phosphoglycerate kinase 1	-4.00	Carbohydrate degradation; glycolysis
2	P29391	Ferritin light chain 1	4.00	Stores iron in a soluble, non-toxic, readily available form. Important for iron homeostasis. Iron is taken up in the ferrous form and deposited as ferric hydroxides after oxidation
3	Q63836	Selenium-binding protein 2	4.00	Bind selenium and acetaminophen
4	P17182	$\alpha$ -enolase	4.00	Multifunctional enzyme that, as well as its role in glycolysis, plays a part in various processes such as growth control, hypoxia tolerance and allergic responses (by similarity). May also function in the intravascular and pericellular fibrinolytic system due to its ability to serve as a receptor and activator of plasminogen on the cell surface of several cell-types such as leukocytes and neurons

after LXT supplement in MRL/*lpr* mice. We initially analyzed the expression profiles of kidney proteins in the MRL/*lpr* model after LXT administration using 2-DE. MS revealed that the protein level of phosphoglycerate kinase 1 was decreased in the MRL/*lpr* group compared with LXT treatment (Table I). Up-regulation of phosphoglycerate kinase 1 in MRL/*lpr* mice after LXT administration indicated an enhanced stress response to the redox status. As shown in Table I, ferritin light chain 1, selenium-binding protein 2 and  $\alpha$ -enolase expression were all significantly increased in the kidney of MRL/*lpr* mice. There was a striking decrease in the level of these proteins in the kidney in MRL/*lpr* mice after LXT administration.

## Discussion

The major finding of this study is the ability of LXT to alleviate the autoimmune signs of SLE in MRL/*lpr* mice. A significant reduction of markers commonly used to monitor murine SLE disease progression was observed for the first time. In particular, our results suggest that part of the beneficial effect of LXT on murine SLE occurs through the modulation of the kidney inflammation that is dependent on the redox status. This was evidenced by enhanced tissue GSH concentration and upregulated expression of the cytoprotective SMP30 protein. LXT immunomodulatory effect was associated with a significant decrease in typical pathological signs (splenomegaly, autoantibodies, and enhanced Th1 and Th2 cytokine expression) compared with untreated MRL/*lpr* mice.

SLE is an autoimmune disease characterized by intrinsic T cell and B cell defects. Previous studies have revealed a deficiency of CD4<sup>+</sup>CD25<sup>+</sup> T cells in (NZB  $\times$  NZW)F1 and (SWR/NZW)F1 lupus-prone mice (16). In the present study, we demonstrated both that the percentage of T cells in

peripheral blood and spleen was significantly decreased in MRL/*lpr* mice compared with normal mice, and that the percentages of Tregs was significantly increased with LXT therapy in SLE animals. In addition, LXT significantly decreased IFN $\gamma$  secretion, indicating that stimulation of T cells could affect the production of Th1 and Th2 cytokines in LXT-treated MRL/*lpr* mice. We report for the first time that, *in vivo*, LXT enhances T cells immunoregulatory function, adding a new dimension to its immunotherapeutic potential. In addition, our studies suggest that the response of T cells to LXT differs significantly from the response seen with other inhibitors of the immune system.

PPAR $\gamma$  expression may be involved in kidney pathology that typically shows severe mesangial proliferation in MRL/*lpr* mouse at 21 weeks of age. As demonstrated by the current experiment, with administration of the Chinese herbal medicine LXT to MRL/*lpr* mice, only mild mesangial proliferation was observed. Despite the use of PPAR $\gamma$  ligands in treating autoimmune disease, no studies have examined the role of T cells in mediating the immunoregulatory effects related to PPAR $\gamma$  expression in the kidneys of MRL/*lpr* mice. In this study, we used the Chinese herbal medicine LXT to characterize the relationship between PPAR $\gamma$  expression and Treg activation.

LXT played a role in the attenuation of SLE in MRL/*lpr* mice. In this study, LXT treatment significantly reduced not only iNOS protein level but also the glomerular IgG deposits in MRL/*lpr* mice. The antiproliferative effects of LXT as the link intracellular oxidative stress was demonstrated in our study. Redox status is important in the regulation of the immune system (17), and GSH homeostasis plays a key role in the treatment of diseases in which cytokines are major participants in the pathophysiology (18). Our findings showing disease-dependent decreases of intracellular GSH concentration in MRL/*lpr* mice are consistent with literature data



indicating decreased blood sulfhydryl or GSH concentrations in T-cells (10) of patients with SLE (9). They also agree with literature data suggesting a defective antioxidant system in MRL/*lpr* mice (19). In this study, LXT's ability to elicit cytoprotective effects by enhanced tissue GSH expression and lower oxidative stress were shown for the first time, and this is in agreement with data showing increased Mn-SOD and SMP30 levels. High levels of SMP30 were maintained in the kidneys of young and adult rats. Later, as aging progressed to senescent stages, amounts of SMP30 significantly decreased in both the liver and the kidney (20). SMP30 evidently plays an important role in  $\text{Ca}^{2+}$  homeostasis and in the modulation of effector molecules including some enzymes required for  $\text{Ca}^{2+}$  to perform its actions (21). Our results suggest that the down-regulation of SMP30 in the kidney may increase the tissue susceptibility for harmful stimuli in MRL/*lpr* tissues and may cause the deterioration of renal functions. The change in SMP30 expression after LXT treatment might account for the attenuation of cellular functions by maintaining calcium homeostasis, and the amelioration of oxidative stress stimuli in MRL/*lpr* mice.

We initially analyzed the expression profiles of kidney tissue in the MRL/*lpr* model after LXT administration using 2-DE.  $\alpha$ -enolase (22) is a glycolytic enzyme, widely distributed in various tissues. Kidney and thymus contain the highest amount of the enzyme (23). The data obtained so far indicate that in SLE nephritis  $\alpha$ -enolase is overexpressed in the kidney and is present in active inflammatory lesions (24). There is an abundance of selenium-binding protein 2 (SBP2), formerly known as 56-kDa APAP-binding protein, in the mouse liver (25) and kidney (26). Ferritin acts as an important regulator of cellular iron alterations in both cell cycle arrest and apoptosis (27). Iron is a potential toxin at elevated tissue concentrations (28), because this extremely reactive free radical species triggers chain reactions that can injure a number of biological targets, mainly lipid structures (29). The present investigation was undertaken to determine if LXT treatment of MRL/*lpr* mice would reduce susceptibility to possible nephrotoxicity in association with reduction of SBP2 and ferritin light chain 1 expression, and restore renal GSH concentration.

As shown in Fig. 8C,  $\alpha$ -enolase, selenium-binding protein 2 and ferritin light chain 1 expression were significantly decreased in the kidneys of MRL/*lpr* mice after LXT administration. The other was that decreased expression of these proteins in the kidney may attenuate oxidative stress networks and reduce inflammation syndrome in SLE mice. Therefore, LXT administration may result in a downstream reduction of both oxidative stress and inflammation in kidney of MRL/*lpr* mice, but further studies are required to elucidate detailed mechanisms.

Phosphoglycerate kinase (PGK) is an mRNA binding protein and is involved in post-transcriptional regulation of urokinase-type plasminogen activator receptor mRNA (30). Deficiency of PGK has been associated with hemolytic anemia, mental retardation, myoglobinuria, and rhabdomyolysis (31,32). Luan *et al* demonstrated that anti-DNA antibodies may cross-react with PGK-1 and decrease PGK mRNA expression (33). These changes may be associated with the hematological (anemia) and neurological (cerebritis) findings in SLE (34). The results of our experiments resembled those

reported previously. The ability of LXT to downregulate serum anti-dsDNA antibody levels is consistent with enhanced PGK protein expression by enhanced stress-response redox status.

In conclusion, this study demonstrated that LXT exerts dramatic immunomodulatory action in MRL-mediated immune dysfunction and kidney injury, and this effect may be caused at least in part by its action on Treg cell expression. This herbal remedy triggered alterations in inflammation and its actions are associated with changes in cellular stress proteins involved in oxidative stress and apoptosis response. Further studies are needed to determine the suitability of LXT treatment for autoimmune pathologies in humans.

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