

Original Article

Curcumin attenuates lupus nephritis in MRL/lpr mice by suppressing macrophage-secreted B cell activating factor (BAFF)

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Abstract: This study aimed to investigate the therapeutic effects and mechanism of action of curcumin against a MRL/lpr lupus model. Eight-week-old female MRL/lpr mice were used to establish the lupus nephritis model. Histologic and immunohistochemical analysis was conducted for lupus nephritis. Anti-dsDNA IgG and BAFF level were detected by ELISA. Cells directly isolated from the spleen were used to detect macrophage subsets and activation status by FACS. Curcumin reduced the total IgG and anti-dsDNA IgG levels in blood and reduced the activation of B cells in MRL/lpr mice. Moreover, curcumin prevented activation of macrophages in MRL/lpr mice. Levels of BAFF in serum, spleens and kidneys were also reduced in curcumin-treated MRL/lpr mice. *In vitro* experiments showed that curcumin reduced the activation of macrophage and led to the decrease of BAFF from them upon toll like receptor (TLR) 4 stimulation. Curcumin attenuates lupus nephritis in MRL/lpr mice by inhibiting macrophages activation and their secreting BAFF, which may be a potential therapeutic candidate for the treatment of SLE.

Keywords: Curcumin, lupus nephritis, macrophage, B lymphocyte stimulator

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disorder with diverse clinical manifestations. Lupus nephritis is the most common type. Lupus nephritis develops in over 60% of adult SLE patients. Lupus nephritis is usually induced by auto-antibody deposition and activation of multiple immune cells which promote kidney injury [1]. Remission of lupus nephritis requires the use of potent immunosuppressive agents or some biologics, with significant adverse effects and frequent relapses. Therefore, a new therapeutic approach that can significantly and efficiently improve lupus nephritis is still necessary.

Lupus nephritis is characterized by loss of B-cell tolerance and the presence of abnormally activated B cells [2], that contribute to lupus nephritis by secreting pathogenic auto-antibodies. B cells are therapeutic targets in lupus

nephritis and one way to modulate function of B cells is inhibition of the B lymphocyte stimulator (BAFF). BAFF is a member of the TNF superfamily that is necessary for B-cell activation [3]. Soluble BAFF are enriched in serum of lupus nephritis patients and in the target organs of MRL/lpr mice [4]. Therapeutic antagonism of BAFF provides a good way of B cell survival and selection.

The major source of BAFF in SLE patients is mostly from activated macrophages [5-7]. A previous study demonstrated that inhibition of BAFF in macrophages ameliorated lupus-like symptoms of MRL/lpr mice [8]. Toll like receptor (TLR) 4 signaling exerts pivotal functions in the pathogenesis of lupus nephritis [9, 10] and BAFF can be produced upon TLR4 stimulation [2, 11]. Upon TLR4 signaling activation, the cytoplasmic region of TLR4 recruits MyD88 linking TLR4 to IL-1R kinase (IRAK) associated with TRAF6. Sequential activation of IRAK4 and

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TRAF6 causes nuclear factor-kappa B (NF- κ B) activation [12], leading to the expression of BAFF.

Curcumin, which comes from a diferuloylmethane present in *Curcuma longa*, is the active component of the spice turmeric with significant anti-inflammatory, antitumor, and anti-oxidant properties [13]. Recent studies have showed that curcumin could attenuate SLE progression by suppressing the differentiation of Tregs [14], and the production of pro-inflammatory cytokines [15]. Interestingly, a previous study showed that curcumin could effectively inhibit rheumatoid arthritis by suppressing the expression of BAFF by influencing NF- κ B signaling pathways [16, 17]. However, it is unclear whether the anti-SLE effect of curcumin is associated with the regulation of BAFF production. Therefore, in this study, the therapeutic effects and mechanism of action of curcumin against MRL/lpr lupus model were investigated.

Materials and methods

Reagents

Curcumin and DMSO were purchased from Sigma Chemical Company (St. Louis, MO, USA). Antibodies against mouse B220-FITC, F4/80-PE, CD86-APC and CD69-APC were purchased from eBiosciences (San Diego, USA). LPS was purchased from Sigma-Aldrich, (St. Louis, USA). ELISA kits for IgG, Anti-dsDNA IgG and Mouse Albumin ELISA Quantitation Set were obtained from Bethyl Laboratories (Montgomery, USA). BAFF ELISA kit was obtained from Abcam, Inc. (Cambridge MA, USA). SYBR Green PCR Master mix was purchased from Applied Biosystems (Foster City, CA). CD45R (B220) beads were obtained from Miltenyi Biotec (Bergisch Gladbach, Germany). Dulbecco's modified Eagle's medium (DMEM), RPMI 1640 and fetal bovine serum (FBS) were purchased from Gibco Invitrogen (Paisley, UK). Antibodies against mouse C3c, BAFF and IgG were purchased from Abcam, Inc. (Cambridge MA, USA). Murine M-CSF was obtained from Miltenyi Biotec (Bergisch Gladbach, Germany). Murine BAFF neutralizing antibody was obtained from R&D Systems (Minneapolis MN, USA). CCK-8 was obtained from DojinDo Molecular Technologies (Kyushu, Japan).

Animals

MRL/lpr mice, which develop a systemic autoimmune disease resembling human SLE characterized by elevated levels of auto-antibodies (IgG and anti-dsDNA), and lupus nephritis, lymphadenopathy, and splenomegaly, were used in this study. Eight-week-old female MRL/lpr mice and C57BL/6 mice were obtained from the Animal Model Research Center of Nanjing University (Nanjing, China) and kept under pathogen-free housing conditions in a 12-h light and dark cycle. MRL/lpr mice were injected intraperitoneally with curcumin (50 mg/kg) on every other day either from 12 week old mice or vehicle-treated mice treated with normal saline (containing DMSO% = 0.5%). All mice were sacrificed by cervical dislocation at 20 weeks of age. All animal studies were approved by Kunming Medical University.

Proteinuria analyses

Proteinuria was collected weekly and the total urinary protein was determined using a Mouse Albumin ELISA Quantitation Set according to the manufacturer's instructions and the urine was applied at dilutions of 1:100.

Histologic and immunohistochemical analysis

Sections (4 μ m) were cut from paraffin-embedded kidney tissue, fixed in formalin (Beyotime, Shanghai, China), and stained with hematoxylin and eosin. Immunohistochemistry was performed on paraffin-fixed kidney sections using primary antibodies for IgG according to the manufacturer's instructions. The antibodies were applied at concentrations ranging from 1:500. Immuno-labeled sections were scanned using an Aperio ScanScope Slide Scanner (BIO-TEK, Vermont, USA).

Plasma cytokines ELISA

Anti-IgG and anti-dsDNA IgG were analyzed by ELISA according to the manufacturer's instructions and the sera were applied at dilutions of 1:100,000 and 1:500,000. Cytokine analysis was performed using mouse BAFF ELISA Kit and according to the manufacturer's instructions. Absorbance was determined using an ELx-800 Universal Microplate Reader (BIO-TEK, Vermont USA).

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Flow cytometric analysis

Spleens were digested to a single-cell suspension with collagenase type D (Roche) (1 mg/ml) and DNase I (Roche) (0.1 mg/ml) in HBSS at 37°C for 30 min, after which red cells were lysed. After washing, cells were immediately processed for flow cytometry.

To detect macrophage subsets and activation status, cells directly isolated from the spleens were pre-incubated with anti-F480-PE (0.6 µl/test), anti-CD69-APC (0.5 µl/test) and anti-CD86-APC (0.5 µl/test). Cells were stained with specific antibodies for 30 min at 4°C in the dark. Cells were then washed with buffer to remove the excess stains and analyzed in a FACS Calibur flow cytometer.

To detect B cell subsets and activation status, cells directly isolated from the spleen were pre-incubated with anti-B220-FITC (1 µl/test), anti-CD69-APC (0.6 µl/test). Cells were stained with specific antibodies for 30 min at 4°C in the dark. Cells were then washed with buffer to remove the excess stains and analyzed in a FACS Calibur flow cytometer.

Cells were stained with specific antibodies for 30 min at 4°C in the dark, then washed with buffer to remove the excess stains and analyzed in a FACS Calibur flow cytometer (Becton Dickinson, San Diego, USA). Labeled cells were analyzed on FACS Calibur flow cytometer and data were analyzed with Flow Jo software.

Cells culture

Mouse spleen B cells were purified using a mouse CD45R (B220) beads according to the recommendations of the manufacturer. Primary mouse bone marrow-derived macrophage (BMDMs) were prepared as described before [18, 19] and maintained in RPMI 1640 medium supplemented with 10% FBS, supplemented with 100 U/ml of penicillin, and 100 mg/ml of streptomycin under a humidified 5% (v/v) CO₂ atmosphere at 37°C.

Cells were pretreated with curcumin in 5 µM, 10 µM, and 20 µM for 2 h, and then stimulated with LPS (100 ng) for 48 h. The expression of co-stimulatory molecules CD69, and CD86 was examined by flow cytometry. For quantification of the production of BAFF, the same treatment

was done, but stimulation with LPS was for 48 h. Cell-free supernatants were collected after 48 h and analyzed by ELISA.

Cell viability assay

The cytotoxicity of curcumin was determined using the Cell Counting Kit-8 (CCK-8) according to the manufacturer's protocol. The average optical density formed in control was taken as 100% viability, and the results were expressed as a percentage of the control.

Real-time PCR

RNAs were extracted using Trizol reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. The relative expression levels against β-actin were calculated by 2^{-ΔΔCt} according to the manufacturer's instructions. The primers for Q-PCR were as follows: β-actin: F primer: 5-TCTGGCACCACACCTTCTA-3, R primer: 5-AGGCATACAGGGACAGCAC-3, BAFF: F primer: 5-TGCCTTGGAGGAGAAAGAGA-3, R primer: 5-GGAATTGTTGGCAGTGT-3.

Statistical analysis

Data were expressed as mean ± SEM of three independent experiments and each experiment included triplicate sets. Statistical analysis was performed using one-way ANOVA followed by Dunnett's test between control group and multiple dose groups. P < 0.05 was considered a significant difference. All statistical calculations were performed using statistical software GraphPad Prism (GraphPad, San Diego, USA).

Results

Curcumin attenuates lupus-like pathology in MRL/lpr mice

The proteinuria levels were decreased after curcumin treatment in MRL/lpr mice (**Figure 1A**). Compared to the vehicle-treated mice, curcumin reduced anti-dsDNA IgG levels in the serum of MRL/lpr mice (**Figure 1B**). Moreover, curcumin also decreased the amounts of IgG (**Figure 1C**), which is known to activate complement in murine lupus nephritis. H&E staining also showed that curcumin significantly inhibited the infiltration of lymphoid cells (P < 0.05) (**Figure 1D**). IgG IHC staining of the kidney tissue revealed significantly deposition of IgG

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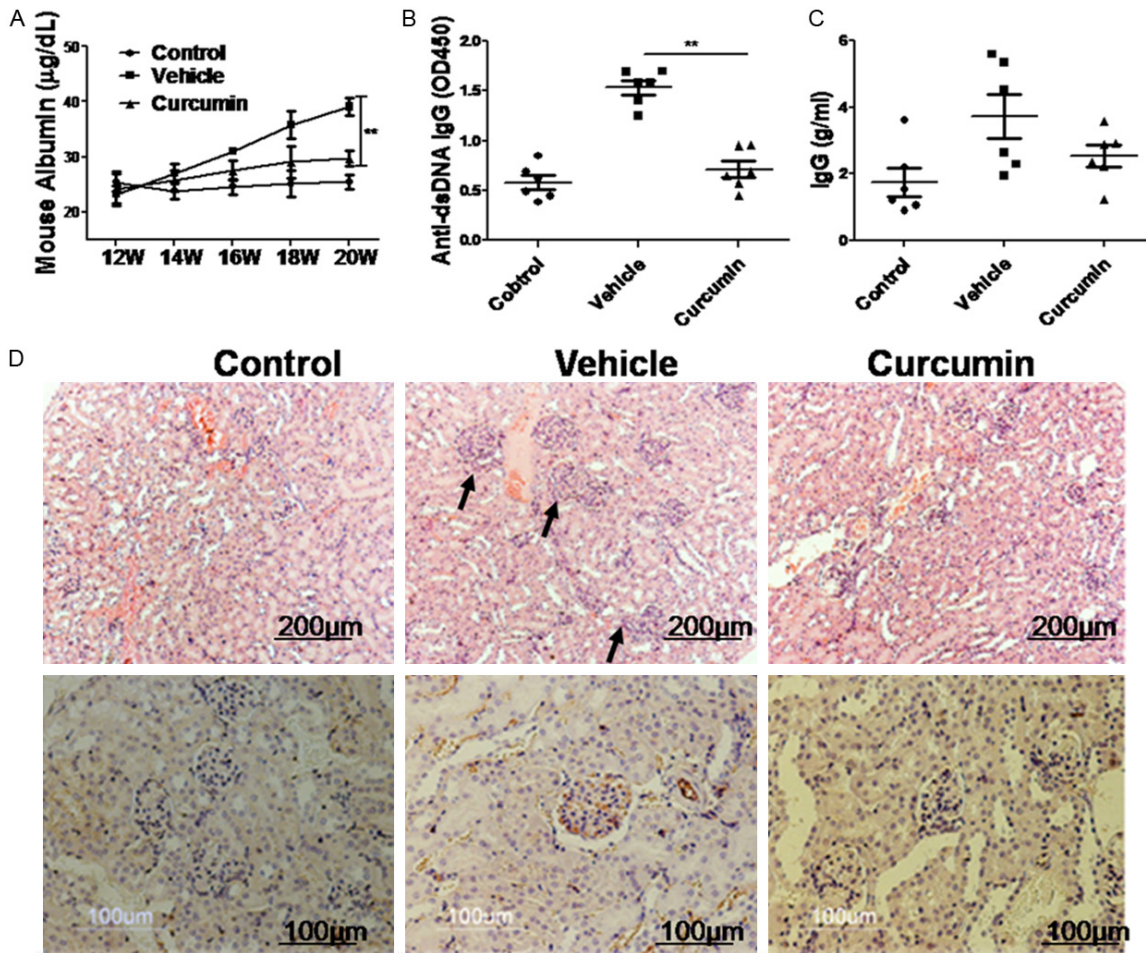


Figure 1. Curcumin attenuates lupus-like pathology in MRL/lpr mice. A. Proteinuria in MRL/lpr mice was determined using Mouse Albumin ELISA Quantitation Set; B. Serum levels of IgG against dsDNA in MRL/lpr mice were determined by ELISA; C. Serum levels of total IgG in MRL/lpr mice were determined by ELISA; D. Kidney sections from MRL/lpr lupus-prone mice showed histologic differences. H&E staining revealed inflammatory infiltrates. IgG deposit was analyzed by IHC staining. The data represent the mean scores \pm SEM ($*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$).

immune complexes, but only occasional staining of capillaries in the glomeruli of vehicle-treated mice. In contrast, there was little or no IgG deposition in the glomeruli of mice treated with curcumin **Figure 1D**).

Curcumin reduces BAFF levels in MRL/lpr mice

Herein, the expression of BAFF in kidney and spleen tissues of treated mice was measured. Curcumin significantly reduced the expression of BAFF in spleens (**Figure 2A**) and kidneys ($P < 0.05$) (**Figure 2B**). Curcumin also significantly reduced production of BAFF in sera of MRL/lpr mice ($P < 0.05$) (**Figure 2C**). Activation of splenic B cells was further determined by flow cytometry. Results showed that curcumin significantly

reduced activation of splenic B cells by decreasing CD69 expression compared with vehicle-treated mice ($P < 0.05$) (**Figure 2D, 2E**).

Curcumin reduces BAFF levels in macrophages in MRL/lpr mice

Activated macrophages produced more BAFF in MRL/lpr mice, and then activation of macrophages in all groups of mice were analyzed by flow cytometry. Results showed that curcumin significantly reduced the expression of splenic macrophages activation maker CD69 and CD86 ($P < 0.05$) (**Figure 3A-C**). Furthermore, curcumin significantly reduced BAFF expression in peritoneal macrophages from MRL/lpr mice ($P < 0.05$) (**Figure 3D**).

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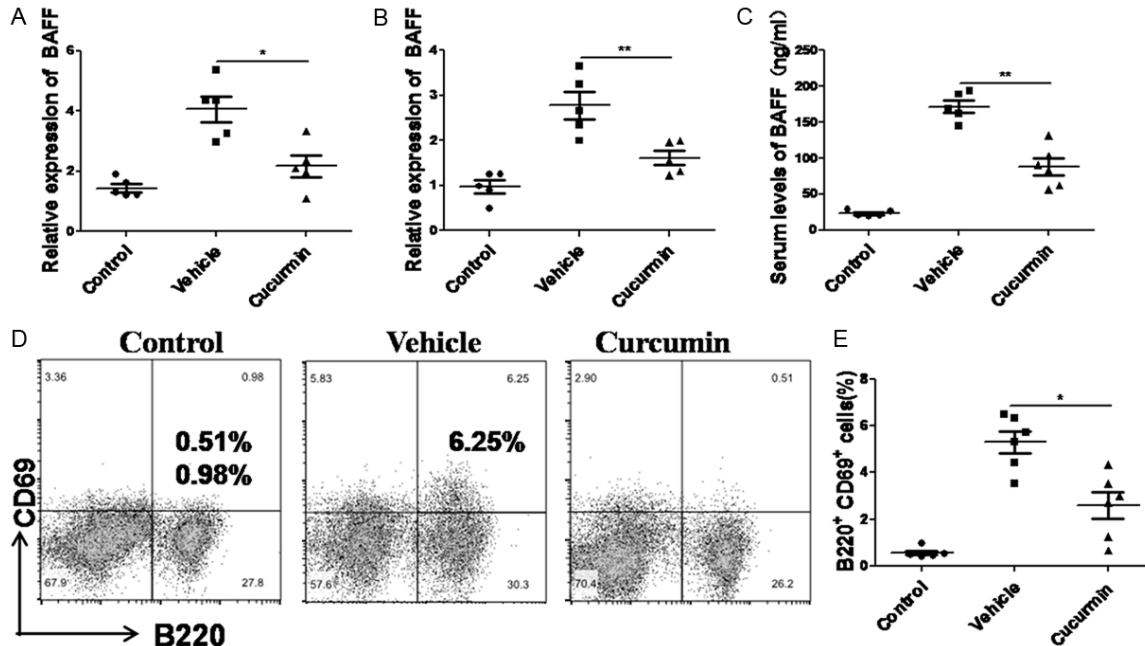


Figure 2. Curcumin reduces BAFF levels in MRL/lpr mice. A, B. Expression of BAFF gene in the spleens and kidneys was measured by quantitative real-time PCR (n = 6); C. Serum levels of BAFF were determined by ELISA (n = 6); D, E. The surface expressions of CD69 in B220⁺ B cells in the spleens were analyzed by flow cytometry (n = 6). Data represent mean scores \pm SEM (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).

Curcumin suppresses activation of BMDMs and BAFF secretion upon TLR4 stimulation

It was pertinent to note that curcumin (1-20 μ M) did not affect the viability of fresh isolated BMDMs significantly as determined by CCK8 assay (Figure 4A). To identify the effect of curcumin on the activation of isolated BMDMs *in vitro*, BMDMs were treated with curcumin at concentrations of 5 μ M, 10 μ M and 20 μ M for 2 hours and then stimulated by LPS. Curcumin significantly reduced the BMDMs activation by decreasing expression of CD69 and CD86 ($P < 0.05$) (Figure 4B, 4C). BAFF expression was also suppressed in activated BMDMs after curcumin-treatment (Figure 4D). Curcumin also significantly reduced the protein levels of BAFF in BMDMs ($P < 0.05$) (Figure 4E). Furthermore, IL-6 expression was also suppressed in BMDMs after curcumin treatment.

Curcumin inhibits B cell activation through reducing BAFF secretion

CD69 expression was significantly decreased under the presence of curcumin-treated BMDMs ($P < 0.05$). Expectedly, BAFF could change this progression (Figure 5A, 5B).

Discussion

SLE is an autoimmune disorder, while lupus nephritis is the most common manifestation in SLE patients. Curcumin is known to possess potent anti-arthritis properties. However, the molecular mechanisms for such efficacy of curcumin are not well known. In the current study, we showed that curcumin inhibits lupus progression in MRL/lpr mice. Furthermore, the improvement of curcumin on lupus resulted from the decrease of BAFF expression in macrophages.

Over the last decades, the key role of BAFF in the pathogenesis of lupus nephritis has come to light [2, 20, 21]. BAFF has been identified as an important cytokine for B cell hyperplasia and autoantibody production in autoimmune diseases [22]. BAFF blockade depletes transitional type 2, follicular, and marginal zone B cells, with sparing of germinal center responses, allowing the generation of high-affinity pathogenic auto-antibodies by somatic mutation [23]. Over-expression of BAFF has been observed in spontaneous murine SLE models and in humans with autoimmune disease. Moreover, BAFF-transgenic mice exhibit a br-

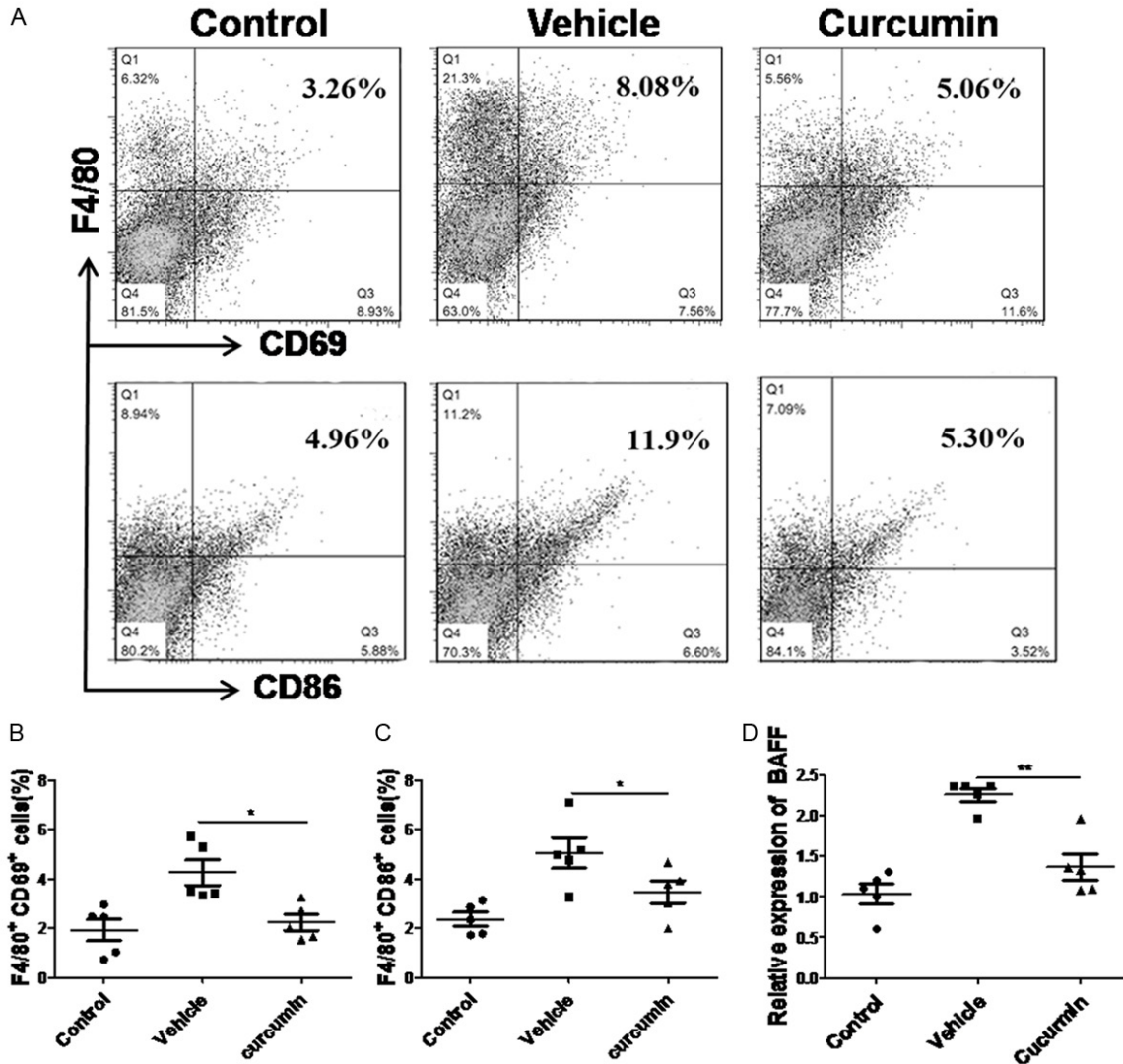


Figure 3. Curcumin reduces BAFF levels in MRL/lpr mice. A-C. The surface expression of CD69 and CD86 in F4/80 macrophages in spleens was analyzed by flow cytometry (n = 6); D. Expression of BAFF gene in the peritoneal macrophages was measured by quantitative real-time PCR (n = 6). Data represent mean scores ± SEM (*P ≤ 0.05, **P ≤ 0.01).

breakdown of B cell tolerance, leading to a SLE-like condition [24, 25]. Therefore, BAFF is considered as a promising therapeutic target for SLE.

BAFF are mainly produced by innate immune cells such as macrophages. Their expression is increased in the presence of IFN-γ, IL-10 and granulocyte colony-stimulating factor (G-CSF) as well as by the activation of TLRs such as TLR4 [26]. Previous research has demonstrated that TLR4 signaling exerts pivotal functions in SLE pathogenesis [27-29]. Exogenous ligands that activate TLR4 in macrophages have been

shown to exacerbate murine SLE by producing BAFF.

The use of natural anti-inflammatory products provides an attractive and safe alternative to conventional methods for modulation of inflammatory disorders. Curcumin is an anti-inflammatory food product that has been used for centuries in traditional medicine [30]. Previous studies have shown that curcumin inhibits rheumatoid arthritis in association with inhibition of inflammatory cytokines, such as BAFF [16]. In addition, many studies also showed curcumin inhibits SLE progression. Curcumin could

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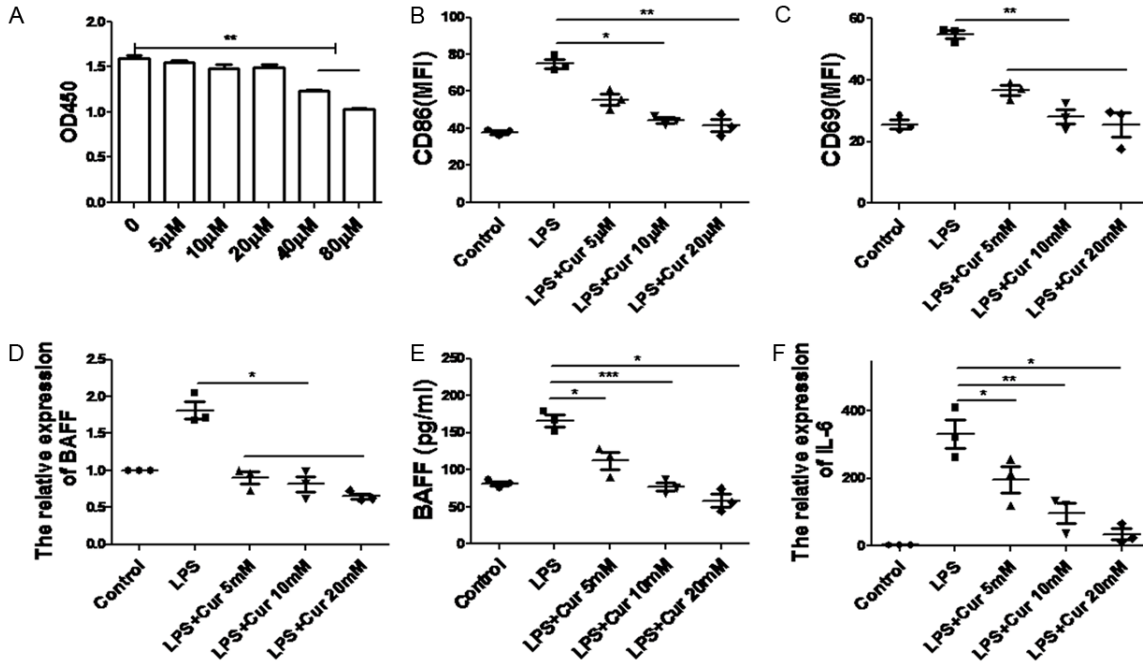


Figure 4. Curcumin suppresses the activation of BMDMs and BAFF secretion. A. BMDMs were treated with various concentrations of curcumin for 24 h and cell viability was measured by CCK-8 assay; B, C. BMDMs were pretreated with curcumin for 2 h before 5 μM, 10 μM and 20 μM stimulation for indicated periods. The surface expression of CD69 and CD86 in BMDMs was analyzed by flow cytometry; D, E. BMDMs were pretreated with curcumin for 2 h before 5 μM, 10 μM, and 20 μM stimulation for 6 h, and BAFF levels were measured by Q-PCR (left). BMDMs were pretreated with curcumin for 2 h before 100 μM, 50 μM and 25 μM stimulation for 48 h, and cell-free supernatants were collected. Protein levels of BAFF in the supernatants were also measured by ELISA; F. BMDMs were pretreated with curcumin for 2 h before 5 μM, 10 μM and 20 μM stimulation for 6 h, and IL-6 levels were measured by Q-PCR (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).

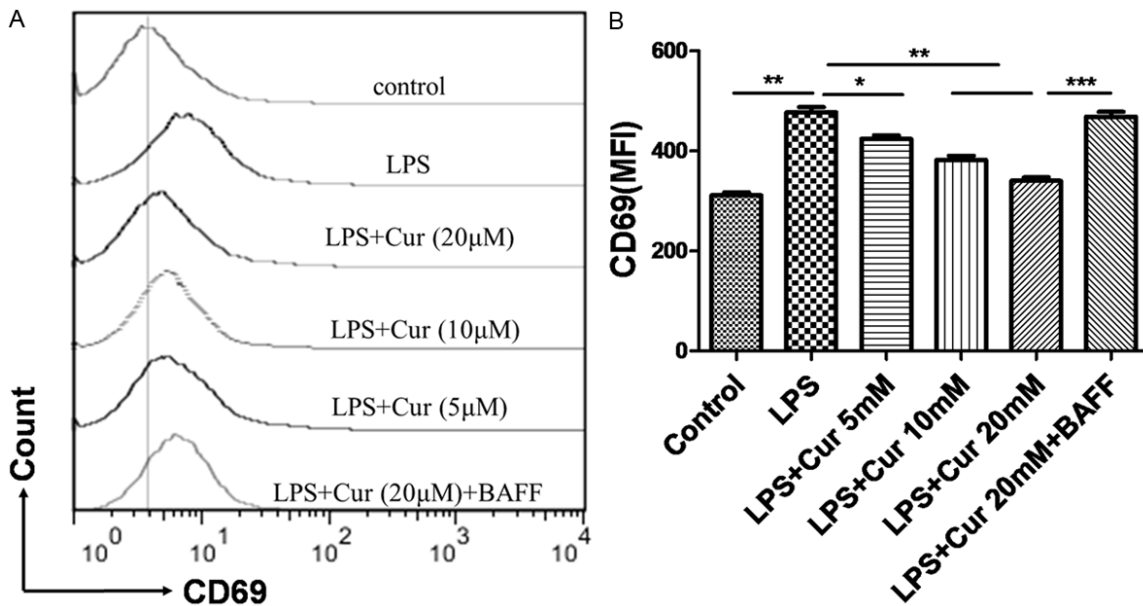


Figure 5. Curcumin inhibits B cells activation through reducing BAFF secretion. A, B. BMDMs were pretreated with curcumin for 2 h before 5 μM, 10 μM and 20 μM stimulation for 48 h, cell-free supernatants were collected. Freshly purified B cells were cultured with the upper supernatants in the presence or absence of BAFF. Activation of B cells was measured using flow cytometry. The surface expression of CD69 in B cells was analyzed by flow cytometry in the BMDMs groups. Data are the mean \pm SEM (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).

modulate Th17/Treg balance specifically in CD4⁺ T cells of SLE patients without affecting healthy subjects [31]. It was showed that curcumin exerted a protective effect against LN in NZB/W F1 mice; moreover, the protective effects of curcumin in LN may involve its interaction with Treg cells [32]. Other work has shown that curcumin could reduce the proliferation of PBMCs in LN patients by inhibiting the activation of PYK2. This ability to modulate PYK2 activation was specifically present only on PBMCs of SLE patients without affecting the PBMCs of healthy subjects [33]. However, there is no study examining the effect of curcumin on BAFF expression in SLE. In this study, we demonstrate that curcumin can inhibit the expression of BAFF *in vivo* and *in vitro* and thus identify BAFF as a novel target molecule for curcumin in SLE.

In conclusion, curcumin markedly ameliorated lupus progression in MRL/lpr mice, which was accompanied by a decrease of BAFF production in serum, spleen, and kidney as well as decrease of activation of B cells. Curcumin also inhibited BAFF production in macrophages. The *in vitro* experiment in macrophages showed that curcumin significantly inhibited LPS-induced BAFF expression. Our study suggests that repression of BAFF production may be a novel mechanism by which curcumin improves SLE.

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This study was approved by The Ethics Committee of The Second Affiliated Hospital of Kunming Medical University. Participants have provided their written informed consent to participate in this study.

Disclosure of conflict of interest

None.

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References

- [1] Talaat RM, Mohamed SF, Bassyouni IH, Raouf AA. Th1/th2/th17/treg cytokine imbalance in systemic lupus erythematosus (sle) patients: correlation with disease activity. *Cytokine* 2015; 72: 146-153.
- [2] Ding H, Wang L, Wu X, Yan J, He Y, Ni B, Gao W, Zhong X. Blockade of B-cell-activating factor suppresses lupus-like syndrome in autoimmune bxs mice. *J Cell Mol Med* 2010; 14: 1717-25.
- [3] Jacob CO, Yu N, Sindhava V, Cancro MP, Pawar RD, Putterman C, Stohl W. Differential development of systemic lupus erythematosus in nzm 2328 mice deficient in discrete pairs of baff receptors. *Arthritis Rheumatol* 2015; 67: 2523-35.
- [4] Davidson A. Targeting baff in autoimmunity. *Curr Opin Immunol* 2010; 22: 732-9.
- [5] Mercado U. B lymphocyte stimulator in systemic lupus erythematosus. *Rev Med Inst Mex Seguro Soc* 2012; 50: 349-350.
- [6] Panchanathan R, Choubey D. Murine baff expression is up-regulated by estrogen and interferons: implications for sex bias in the development of autoimmunity. *Mol Immunol* 2013; 53: 15-23.
- [7] Stohl W, Hilbert DM. The discovery and development of belimumab: the anti-blys-lupus connection. *Nat Biotechnol* 2012; 30: 69-77.
- [8] Zhou L, Sun L, Wu H, Zhang L, Chen M, Liu J, Zhong R. Oridonin ameliorates lupus-like symptoms of mrl(lpr/lpr) mice by inhibition of B-cell activating factor (baff). *Eur J Pharmacol* 2013; 715: 230-7.
- [9] Lee TP, Tang SJ, Wu MF, Song YC, Yu CL, Sun KH. Transgenic overexpression of anti-double-stranded DNA autoantibody and activation of toll-like receptor 4 in mice induce severe systemic lupus erythematosus syndromes. *J Autoimmun* 2010; 35: 358-67.
- [10] Bezalel S, Asher I, Elbirt D, Stoeberl ZM. Novel biological treatments for systemic lupus erythematosus: current and future modalities. *Isr Med Assoc J* 2012; 14: 508-14.
- [11] Moon EY, Lee JH, Lee JW, Song JH, Pyo S. Ros/epac1-mediated rap1/nf-kappab activation is required for the expression of baff in raw264.7 murine macrophages. *Cell Signal* 2011; 23: 1479-88.
- [12] Ko HJ, Yang JY, Shim DH, Yang H, Park SM, Curtiss R 3rd, Kweon MN. Innate immunity mediated by myd88 signal is not essential for in-

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- duction of lipopolysaccharide-specific B cell responses but is indispensable for protection against salmonella enterica serovar typhimurium infection. *J Immunol* 2009; 182: 2305-12.
- [13] Ji JL, Huang XF, Zhu HL. Curcumin and its formulations: potential anti-cancer agents. *Anti-cancer Agents Med Chem* 2012; 12: 210-8.
- [14] Lee H, Kim H, Lee G, Chung HS, Bae H. Curcumin attenuates lupus nephritis upon interaction with regulatory T cells in new zealand black/white mice. *Br J Nutr* 2013; 110: 69-76.
- [15] Kurien BT, Harris VM, Quadri SM, Coutinho-de Souza P, Cavett J, Moyer A, Ittiq B, Metcalf A, Ramji HF, Truong D, Kumar R, Koelsch KA, Centola M, Payne A, Danda D, Scofield RH. Significantly reduced lymphadenopathy, salivary gland infiltrates and proteinuria in mrl-lpr/lpr mice treated with ultrasoluble curcumin/turmeric: increased survival with curcumin treatment. *Lupus Sci Med* 2015; 2: e000114.
- [16] Huang G, Xu Z, Huang Y, Duan X, Gong W, Zhang Y, Fan J, He F. Curcumin protects against collagen-induced arthritis via suppression of baff production. *J Clin Immunol* 2013; 33: 550-7.
- [17] Huang G, Yang Y, Xu Z, Zhou P, Gong W, Li Y, Fan J, He F. Downregulation of B lymphocyte stimulator expression by curcumin in B lymphocyte via suppressing nuclear translocation of NF-kappaB. *Eur J Pharmacol* 2011; 650: 451-7.
- [18] Powell DA, Roberts LM, Ledvina HE, Sempowski GD, Curtiss R 3rd, Frelinger JA. Distinct innate responses are induced by attenuated salmonella enterica serovar typhimurium mutants. *Cell Immunol* 2016; 299: 42-9.
- [19] Larochelle A, Bellavance MA, Michaud JP, Rivest S. Bone marrow-derived macrophages and the CNS: an update on the use of experimental chimeric mouse models and bone marrow transplantation in neurological disorders. *Biochim Biophys Acta* 2016; 1862: 310-22.
- [20] Krivosikova M, Dallos T, Maslinski W, Buc M. B cell activating factor, its role in autoimmunity, and targeting in autoimmune diseases. *Bratisl Lek Listy* 2009; 110: 137-45.
- [21] Ramanujam M, Bethunaickan R, Huang W, Tao H, Madaio MP, Davidson A. Selective blockade of baff for the prevention and treatment of systemic lupus erythematosus nephritis in nzm-2410 mice. *Arthritis Rheum* 2010; 62: 1457-68.
- [22] Powell DA, Roberts LM, Ledvina HE, Sempowski GD, Curtiss R 3rd, Frelinger JA. Distinct innate responses are induced by attenuated salmonella enterica serovar typhimurium mutants. *Cell Immunol* 2016; 299: 42-9.
- [23] Becker-Merok A, Nikolaisen C, Nossent HC. B-lymphocyte activating factor in systemic lupus erythematosus and rheumatoid arthritis in relation to autoantibody levels, disease measures and time. *Lupus* 2006; 15: 570-6.
- [24] Stohl W, Xu D, Kim KS, Koss MN, Jorgensen TN, Deocharan B, Metzger TE, Bixler SA, Hong YS, Ambrose CM, Mackay F, Morel L, Putterman C, Kotzin BL, Kalled SL. Baff overexpression and accelerated glomerular disease in mice with an incomplete genetic predisposition to systemic lupus erythematosus. *Arthritis Rheum* 2005; 52: 2080-91.
- [25] Mackay F, Figgett WA, Saulep D, Lepage M, Hibbs ML. B-cell stage and context-dependent requirements for survival signals from baff and the B-cell receptor. *Immunol Rev* 2010; 237: 205-25.
- [26] Mackay F, Schneider P. Cracking the baff code. *Nat Rev Immunol* 2009; 9: 491-502.
- [27] Jiang W, Gilkeson G. Sex differences in monocytes and tlr4 associated immune responses; implications for systemic lupus erythematosus (sle). *J Immunother Appl* 2014; 1: 1.
- [28] Huang X, Xie Z, Liu F, Han C, Zhang D, Wang D, Bao X, Sun J, Wen C, Fan Y. Dihydroartemisinin inhibits activation of the toll-like receptor 4 signaling pathway and production of type I interferon in spleen cells from lupus-prone mrl/lpr mice. *Int Immunopharmacol* 2014; 22: 266-72.
- [29] Liu Y, Yin H, Zhao M, Lu Q. Tlr2 and tlr4 in autoimmune diseases: a comprehensive review. *Clin Rev Allergy Immunol* 2014; 47: 136-147.
- [30] Anand P, Sundaram C, Jhurani S, Kunnumakara AB, Aggarwal BB. Curcumin and cancer: an "old-age" disease with an "age-old" solution. *Cancer Lett* 2008; 267: 133-64.
- [31] Handono K, Pratama MZ, Endharti AT, Kalim H. Treatment of low doses curcumin could modulate Th17/Treg balance specifically on CD4⁺ T cell cultures of systemic lupus erythematosus patients. *Cent Eur J Immunol* 2015; 40: 461-9.
- [32] Lee H, Kim H, Lee G, Chung HS, Bae H. Curcumin attenuates lupus nephritis upon interaction with regulatory T cells in New Zealand black/white mice. *Br J Nutr* 2013; 110: 69-76.
- [33] Wang M, Zhou G, Lv J, Zeng P, Guo C, Wang Q. Curcumin modulation of the activation of PYK2 in peripheral blood mononuclear cells from patients with lupus nephritis. *Reumatologia* 2017; 55: 269-275.