Heliyon 10 (2024) e26022

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

The mechanism of Langchuangding in treatment of systemic lupus erythematosus via modulating TLR7-IRF7-IFNα pathway

Meijiao Wang [1], Yiyang Zhang [1], Yingqi Zhai [1], Haichang Li, Zhijun Xie [**], Chengping Wen [*]

Research Institute of Chinese Medicine Clinical Foundation and Immunology, School of Basic Medical Sciences, Zhejiang Chinese Medical University, Hangzhou, 310053, Zhejiang, China

ARTICLE INFO

Keywords:
SLE
LCD
TLR7-IRF7-IFNα pathway

ABSTRACT

Object: Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by aberrant activity of the immune system. Plasmacytoid dendritic cells (pDCs) which the main producer of activated type I interferon, are related to SLE disease activity. To investigate the mechanism of Langchuangding (LCD) improving SLE based on TLR7-IRF7-IFNα pathway.

Methods: SLE patients were randomly divided into Chinese medicine combined with western medicine (CWM) group and western medicine (WM) group, to observe the effect of LCD. The percent of pDCs in peripheral blood of SLE patients were detected by flow cytometry, and the influence of LCD on gene expression in SLE patients were detected by gene microarray. Mouse bone marrow cells were differentiated into dendritic like cells (DLC), then divided into Blank, immune complex (IC), LCD and dexamethasone (DXM) group. Employed RT-qPCR to detect *MyD88*, and *IRF7* mRNA, and western blotting to determinate TLR7, MyD88, and p-IRF7 proteins. The IFNα in SLE patients were detected by enzyme-linked immunosorbent assay (ELISA). Employ dual luciferase to observe the interferon stimulated response element (ISRE) gene.

Results: pDCs in WM group was higher than that of CWM group. The plasma IFNα in CWM group was significantly lower than that in WM group. The gene microarray showed that the gene expression of IFNα related signaling pathway in peripheral blood mononuclear cell (PBMC) and genes related to activation and proliferation of immune cells were down-regulated after LCD treatment. The DLCs *MyD88*, and *IRF7* mRNA were down-regulated, TLR7, MyD88, and p-IRF7 proteins were significantly reduced, and the supernatant IFNα was significantly decreased in LCD group. LCD were mildly inhibited activation of ISRE in 293T cells.

Conclusions: In certain degree, LCD is beneficial to SLE patients. LCD therapy SLE may be through TLR7 signaling pathway, and IRF7 may be a promising therapeutic target for the treatment of SLE.

* Corresponding author.
** Corresponding author.
E-mail addresses: xzj575@163.com (Z. Xie), chengpw2010@126.com (C. Wen).
[1] These authors contributed equally to this work as co-first authors.

https://doi.org/10.1016/j.heliyon.2024.e26022
Received 3 January 2024; Received in revised form 5 February 2024; Accepted 6 February 2024
Available online 17 February 2024
2405-8440/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by abnormal immune function leading to variable clinical symptoms, pathogenic autoantibodies, and immune complexes, involving multiple organ systems, and damaging to the health and quality of life of patients. Glucocorticoids (GC) are the first-line drugs for remission treatment. Whereas, long-term use of GC leads to metabolic disorders, secondary infections, cardiovascular disease, and organ damage [1].

SLE belongs to yin-yang toxicity, red butterfly sores, Bi syndrome, and others, according to traditional Chinese medicine (TCM). Langchuangding formula (LCD, also called Jiedu-Quyu-Ziyin fang) is derived from "Shengma Biejia Tang", and play a great role in clearing heat and detoxifying, promoting blood circulation and removing blood stasis, and nourishing yin and kidney. LCD contains ten traditional Chinese herbs and the effectiveness in treating SLE support by more than a decade of clinical practice [2,3]. It can significantly reduce the lupus disease activity score, decrease the incidence of infection, alleviate the adverse effects of GC, and achieve the effect of synergism and toxicity-reducing [4].

Type I interferon (IFN I) pathway plays a critical role in SLE pathogenesis [5]. Plasmacytoid dendritic cell (pDC) accounts for only 0.2%–0.8% of PBMC in healthy individuals, and is the main producer of activated IFN I in response to single-stranded RNA or double-stranded DNA (dsDNA) stimulation [6,7]. Furthermore, pDCs secrete proinflammatory chemokines and cytokines, such as interleukin-6 (IL-6) and tumour necrosis factor. pDCs are a key pathogenic cells in autoimmune diseases, and are related to SLE disease activity [8]. However, there were few studies on the effect and mechanism of LCD on pDC. In this study, we evaluated the mechanism of LCD modulating TLR7-IRF7-IFNα pathway in pDC.

2. Materials and methods

2.1. SLE participants in clinic

Clinical patients were met the 2009 SLICC modified ACR Classification criteria for systemic lupus erythematosus [9] with mild to moderate activity ($5 \leq$ systemic lupus erythematosus disease activity index (SLEDAI) ≤ 14). The ages of female SLE patients were ranged from 14 to 50. SLE patients received glucocorticoid therapy (dosage ≥ 5 mg, dosage converted by prednisone dosage), not received any other immunosuppressant, and any TCM treatment within one month. Excluded patients who pregnancy or with other autoimmune diseases or chronic inflammatory diseases. This study was approved by the Medical Ethics Committee of Zhejiang Chinese Medical University (2015zjtcm-016), and obtained informed consent of the participants.

2.2. Preparation of LCD

The LCD formula comprised ten herbs in proportions, as follows: Gan Di Huang (*Rehmannia glutinosa* (Gaert.) DC), Zhi Bie Jia (*Trionyx sinensis* Wiegmann), Sheng Ma (*Actaea cimicifuga* L. (syn. *Cimicifuga foetida* L.)), Bai Hua She She Cao (*Scleromitrion diffusum* (Willd.) R. J. Wang (syn. *Hedyotis diffusa* Willd.)), Qing Hao (*Artemisia annua* L.), Ji Xue Cao (*Centella asiatica* (L.)), Chi Shao (*Paeonia anomala*subsp. *veitchii* (Lynch) D. Y. Hong, and K. Y. Pan), Yi Yi Ren (*Coix lacryma-jobi* L. *var.mayuen* (Roman.) Stapf), Fo Shou (*Citrus medica* L.), and Sheng Gan Cao (*Glycyrrhiza uralensis* Fisch. ex DC.). The contents of Chinese medicine (CM) are shown in Supplementary Table 1. All the CM were soaked in water for 1 h. Zhi Bie Jia was decocted for 30min before other herbs were added. The mixture was heated to boil, and maintained microboiling for 1 h. The liquid was filtered, and the CM was decocted for another time. Then mixed the decoction thoroughly, and divided into two parts evenly. The enrolled patients taken one part of the mixed liquid each morning and evening.

2.3. Clinical patient grouping

60 SLE patients were randomly divided into Chinese combined with western medicine (CWM) group and western medicine (WM) group according to their disease conditions, with 30 cases in each group. The baseline information including age, course of disease, and SLEDAI score was collected before treatment. The patients in CWM group or WM group were treated with LCD and prednisone (Pred) or Pred respectively for 4 weeks. Pred dosage were prescribed according to the disease activity, 0.2 mg/kg·d $\leq$ Pred $\leq$ 0.5 mg/kg·d for mild activity patients, and 0.5 mg/kg·d $<$ Pred $\leq$ 1 mg/kg·d for moderately active. We evaluated SLEDAI score after treatment, and fasting venous blood 7 mL were collected from each group in the morning, respectively.

2.4. Flow cytometry

The percentage of pDC in peripheral blood of SLE patients were detected by flow cytometry. Centrifuged blood of SLE patients to obtain plasma and substratum blood cells. Peripheral blood mononuclear cell (PBMC) was isolated by Ficoll density gradient centrifugation (Tianjin Hao Yang Biological Products Technology Co., LTD HY2015). Approximately 10^6 cells were incubated with antibodies against the markers at 4 °C for 30 min protected from light for cell staining. The following antibodies were used: PE/Cyanine 5 anti-human CD11c antibody (301610), FITC anti-human CD123 antibody (306014), PE anti-human CD303 (BDCA-2) antibody (354204). All the antibodies were purchased from Biolegend. pDCs ($CD11c^{-}CD123^{+}CD303^{+}$) in all samples were detected by Beckman Flow Cytometer (Cytomics FC500) and analyzed by FlowJo software.

2.5. LCD-treated rat serum preparation

Ten herbs mentioned above were purchased from the Zhejiang Chinese Medical University Traditional Chinese Medicine Co. Ltd. (Hangzhou, China), prepared, concentrated to 4 g/mL using a rotary evaporator at 50 °C and HPLC analyzed as previously described [10]. Twenty SPF-grade male 8-week-old SD rats were randomly divided into two group, blank group and LCD group, which were administrated normal saline or LCD via gastrogavage respectively, 0.1 g/kg·d for 7 days. 30 min after the last administration, the rats anaesthetized by intraperitoneal injection with 3% pentobarbital sodium (0.2 mL/100 g). The blood of the LCD group and blank group were sterilely collected separately through the celiac vein, then the rats were euthanized with carbon dioxide. After settling for 30min at room temperature, the medicated serum (MS) and the blank serum (BS) were separated by centrifugation at 3000 rpm at 4 °C for 15 min, inactivated at 56 °C for 30 min, and then stored at −80 °C.

2.6. Detect the effect of LCD on gene expression in SLE patients by gene microarray

The blood of SLE patients were obtained in the early morning before treatment, and isolated PBMC. The PBMC were divided into two groups, blank group and LCD group, which were administrated 10% BS or 10% MS, respectively. The PBMC in two groups were cultivated in RPMI 1640 (penicillin 1×10^5U/L, and streptomycin 100 mg/L). The PBMC were cultivated for 24 h and collect in 1 mL RNAiso Plus for gene microarray.

2.7. Determined the level of interferon α (IFNα) in SLE patients by enzyme-linked immunosorbent assay (ELISA)

Employed Human Interferon α (IFNα) ELISA Kit (CUSABIO BIOTECH CO., LTD. S04016407) to detect the IFNα in the plasma of SLE patients according to the manufacturer's protocols.

2.8. Prepared for DLCs

20 SPF-grade female 7-week-old C57BL/6 mice were obtained and fed in the experimental animal centre of Zhejiang Chinese Medical University [SCXK(HU)2017-0005], Zhejiang Province, P.R. China. After one week adaptive feeding, the mice were euthanized with carbon dioxide, and the femur and tibia were obtained immediately in sterile environment. Flushed the bone marrow using serum-free RPMI 1640 medium, dissociated into a single-cell suspension by 70 μm filter, and centrifuged to collect the cells. Adjusted the cell density to about 10^7/mL with phosphate buffer saline. The lymphocyte in bone marrow were separated by mice bone marrow lymphocyte isolation solution (Tianjin Hao Yang Biological Products Technology Co., TBD2013LM). The lymphocyte was adjusted to 10^6/mL by RPMI 1640 medium (10%FBS, penicillin 1×10^5U/L, and streptomycin 100 mg/L). The lymphocyte differentiated to DLCs by tumour necrosis factor α (TNFα, Pepro Tech, 25 ng/mL) and phorbol-12-myristate-13-acetate (PMA, Sigma, 25 ng/mL) for 72 h, and identified by flow cytometry (Supplementary Fig. 1). The animal study was approved by the Laboratory Animal Management and Ethics Committee of the Zhejiang Chinese Medical University (IACUC-20181224-15).

2.9. Prepared for dexamethasone (DXM)

DXM sodium phosphate injection (Guangzhou Pharmaceutical, H44022091) was diluted into 0.02 mg/mL by RPMI 1640 medium.

2.10. Prepared for immune complex (IC)

The serum of SLE patients was used to prepare immune complex according to Terry K. Myers method [11]. Immune complex was dissolved to 50 mg/mL by RPMI 1640 medium, and filtrated by 0.22 μm filter.

2.11. Cell culture and intervention

DLCs were cultured in RPMI 1640 medium (10%FBS, penicillin 1×10^5U/L, and streptomycin 100 mg/L), 37 °C, 5% CO_2 with saturated humidity. DLCs were divided into four groups, blank group, IC group, LCD group and DXM group. The blank group were cultured normally. IC group were added IC (0.5 mg/mL) for 18 h, and then cultured normally for 12 h. LCD group were added IC (0.5

Table 1
Primers for RT-qPCR.

Primers		Sequence 5′-3′
Myd88	Forward	CGGAACTTTTCGATGCCTTTAT
	Reverse	CACACACAACTTAAGCCGATAG
Irf7	Forward	GTGCTGTTTGGAGACTGGCTATTG
	Reverse	ATCCCTACGACCGAAATGCTTCC
Gapdh	Forward	ATCCGTAAAGACCTCTATGCCAACA
	Reverse	GCCGTGGAGTACGACAA

mg/mL) for 18 h and 10% MS for 12 h. The DXM group were added IC (0.5 mg/mL) for 18 h and DXM (10 ng/mL) for 12 h.

2.12. The real-time reverse transcription polymerase chain reaction (RT-qPCR)

Collected DLCs and employed RT-qPCR to detect *MyD88* and *Irf7* mRNA levels. Extract total RNA in DLCs by RNAiso Plus (TaKaRa, 9109), and reverse transcription (TaKaRa, RR036A) and qPCR (Bio-Rad, 172–5124) were performed according to the manufacturer's instructions. The primers were designed and synthesized by Sangon Biotech (Shanghai, China). The PCR program was as follow: 94 °C 300 s, 94 °C 30 sec 54°C 15 s for 35 cycles, 72 °C 300sec. Sequences of the primers were showed in Table 1.

2.13. Protein expression detection via western blotting

DLCs were homogenized in RIPA buffer (Beyotime Biotechnology, P0013B) supplemented with phosphatase and protease inhibitors (Beyotime Biotechnology, P1046), and separated by SDS-PAGE and immunoblotted with antibodies against TLR7 (1:500 dilution, Sigma, WH0051284M4-100UG), MyD88 (1:1000 dilution, abcam, ab2064), p-IRF7(Ser 471/472) (1:1000 dilution, CST, 5184S) and β-actin (1:5000 dilution, Bioker, BK7018).

2.14. Determined the level of IFNα in the supernatant of DLCs by ELISA

IFNα in the supernatant of DLCs were detected by the ELISA Kit (Shanghai Xinfan Biotechnology Co. LTD, XF2357B) according to the manufacturer's protocols.

2.15. Dual-luciferase for interferon stimulated response element (ISRE)

pISRE-TA-luc plasmid was transferred into 293T cells by Lipo 3000 transfection reagent (ThermoFisher, L3000015). After transfect for 24 h, IC was added and co-incubated with the cells for 24 h. Then the corresponding drug was added for 18 h, and groups were shown in Table 2. The cells were collected, and lysed. Employed dual-luciferase reporter assay system (Promega, E1910) to measure the effect on IRF7 transcriptional activity.

2.16. Statistical analysis

The results are presented as the means ± standard deviations and were analyzed by SPSS18.0 for windows. The *t*-test was used for comparison between two groups. Oneway ANOVA was used to determine the statistical significance of differences between the values of various experimental group. Employ GraphPad Prism 8 to visualize. $P < 0.05$ was considered significant.

3. Results

3.1. Clinic information of SLE patients

We collected the clinic information of SLE patients, including age, disease course, and SLEDAI. There was no difference in the baseline in Chinese combined with western medicine group and western medicine group. The age, disease course, and SLEDAI in the two groups before treatment were similar. Whereas, SLEDAI were significantly decreased in the two groups after treatment (Table 3).

3.2. pDCs in SLE patients

Employed flow cytometry to detect the proportion of pDCs ($CD11c^{-}CD123^{+}CD303^{+}$) in SLE patients. It was showed that pDCs in SLE in Chinese combined with western medicine group was significantly lower than that of western medicine group (Fig. 1).

Table 2
293T cell grouping.

Groups	Culture Condition
Blank group	BS 10%
IC group	IC 0.05%
LCD group	Virus+10%MS
DXM group	Virus + DXM 1 μM

IC: immune complex, LCD: Langchuangding, BS: blank serum, MS: medicated serum, DXM: dexamethasone.

Table 3
Clinical data of SLE patients.

	SLE patients		*P* value
	CWM group (n = 30)	WM group (n = 30)	
Age (year) ($\bar{x}$ ±s)	34.03 ± 11.33	36.67 ± 12.02	0.39
Disease Course (year)	5.57 ± 5.65	4.25 ± 6.11	0.39
SLEDAI ($\bar{x}$ ±s)			
Before the treatment	9.87 ± 2.49	9.47 ± 2.84	0.56
After the treatment	7.87 ± 2.04**	7.73 ± 2.24**	0.81

SLEDAI: systemic lupus erythematosus disease activity index, CWM group: Chinese combined with western medicine group, WM group: Western medicine group. Comparison with before the treatment, $^{**}P < 0.01$.

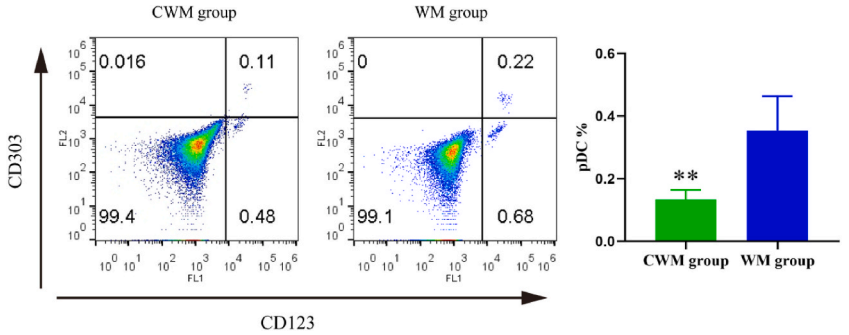

Fig. 1. The proportion of pDCs ($CD11c^{-}CD123^{+}CD303^{+}$) in SLE patients. Cumulative data from the pDCs were shown 5 individuals per group. Results were the mean ± SEM. Comparison with WM group, $^{**}P < 0.01$, by paired 2-tailed *t*-test. CWM group: Chinese combined with western medicine group, WM group: western medicine group.

3.3. Gene microarray results

After LCD treatment, genes associated with IFNα signaling pathways (USP18, IFIT2, IFIT1, IFIT3) of PBMC in SLE patients were down-regulated. The genes related interleukin (IL)-1, IL-4, IL-6 secretion were reduced, genes related dendritic cells, T lymphocytes and other immune cell activation and proliferation, were down-regulated. The genes (IFIT1, P2RX7) related to response to dsRNA were down-regulated. However, the genes (TSPAN32, IFIT1, NLRP3, PYCARD, IFIT2, IFIT3) related to antiviral were decreased. The genes related to apoptosis, programmed death and leukocyte differentiation were up-regulated (Supplementary Fig. 2, Supplementary Table 2, Supplementary Table 3).

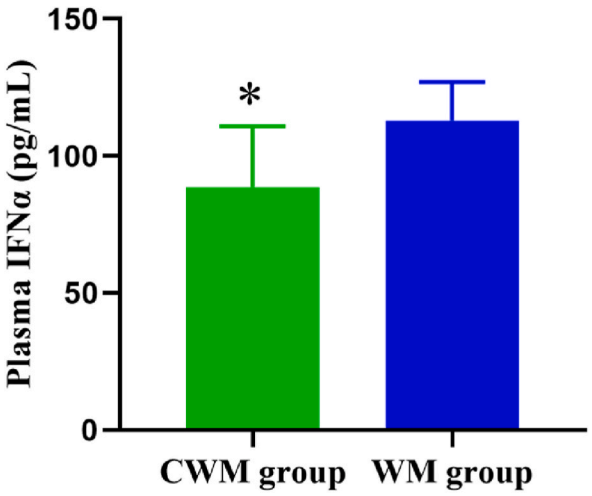

Fig. 2. Plasma IFNα levels in SLE patients. Comparison with WM group, $^{*}P < 0.05$. IFNα: interferon α, CWM group: Chinese combined with western medicine group, WM group: western medicine group.

3.4. LCD and western medicine treatment decreased IFNα levels in SLE patients

According to the gene microarray results, we detected plasma IFNα in the SLE patients. It was showed that plasma IFNα in the Chinese combined with western medicine group was markedly lower than that in the western medicine group (Fig. 2).

3.5. LCD treatment notably reduce Myd88 and Irf7 mRNA in DLCs

It was showed that IC was significantly up-regulated *Irf7* mRNA in DLCs, whereas, has no obvious effect on *MyD88* mRNA expression. Compared with IC group, the *MyD88*, and *Irf7* mRNA were markedly down-regulated in LCD and DXM groups (Fig. 3A and B).

3.6. LCD treatment notably decreased relevant protein in TLR7-pIRF7 pathway in DLCs

It was showed that IC could increased TLR7, and MyD88 protein, and phosphorylation of IRF7 protein in TLR7-pIRF7 pathway in DLCs (Fig. 4A, B, C). Compared with IC group, LCD treatment notably reduced TLR7, and MyD88 proteins, and phosphorylation of IRF7 protein (Fig. 4A, B, C), and DXM treatment significantly decreased TLR7, and MyD88 proteins (Fig. 4A and B), and slightly reduced phosphorylation of IRF7 protein in TLR7-pIRF7 pathway (Fig. 4C).

3.7. LCD treatment remarkably reduced IFNα level in the supernatant of DLCs

The IFNα in supernatant of DLCs was detected by ELISA. It indicated that IC could significantly increased IFNα in the supernatant. Compared with IC group, LCD and DXM treatment remarkably reduced IFNα level in the supernatant (Fig. 5).

3.8. LCD treatment reduced ISRE activity

The result in dual-luciferase showed that IC can mightily activate ISRE as a stimulus in 293T cells, and LCD was mildly inhibited activation of ISRE, and DXM was markedly decreased activation of ISRE (Fig. 6).

4. Discussion

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease character by the production of autoantibodies, which cause inflammation and damage to various organs and tissues. The pathological mechanisms of SLE is unknown, commonly believed to be relative to genetic, environmental, and hormonal factors. Treatment for SLE typically involves medication to control the disease and relieve the symptoms. The commonly used drugs include glucocorticoids (GC), antimalarials, and disease-modifying antirheumatic drugs. In some cases, immune suppressants or biologic agents may be necessary. GCs are the first-line therapy for SLE internationally, alleviate lupus symptoms to some extent, whereas, the use of long-term and high-dose GC leads to many side effects, such as infections and osteonecrosis. LCD has been used clinically in China to enhance the curative effect of GCs in SLE treatment [2–4]. The previous studies showed that LCD may affect the proliferation and activation of B cells by inhibiting the AKT/mTOR/c-Myc signaling pathway to relieve SLE [10], increased prednisone efficacy by increasing Nrf2 expression for the treatment of lupus nephritis [12], possibly inhibit TLR9/MyD88 signaling and promotion of cholesterol efflux to therapy ApoE$^{-/-}$ mice with pristane-induced lupus-like diseases and atherosclerosis [13], and inhibit CD70 gene expression in SLE patients by promoting the DNA methylation of CD70 gene promoter [14].

pDC accounts for only 0.2%–0.8% of PBMC in healthy individuals, and is the main producer of activated type I interferon. pDC abnormal function can cause autoimmune diseases and play a crucial role in the pathogenesis of SLE [15], and were decreased in SLE patients, whereas, accumulated in inflammatory tissues and target organs (such as diseased skin or kidney) [16,17]. pDC in peripheral

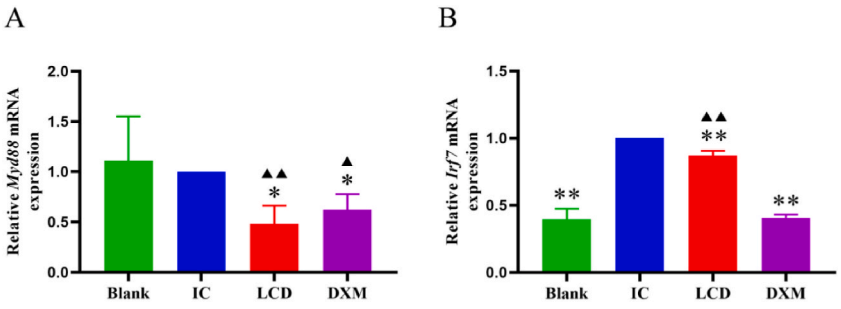

Fig. 3. The relative expression of *MyD88* and *Irf7* mRNA in DLCs. A The relative *MyD88* mRNA level in the DLCs. B The relative *Irf7* mRNA level in the DLCs. *Gapdh* was used as the internal control gene. Comparison with IC group, $^{*}P < 0.05$, $^{**}P < 0.01$, comparison with Blank group, $^{▲}P < 0.05$, $^{▲▲}P < 0.01$. IC: immune complex, LCD: Langchuangding, DXM: dexamethasone.

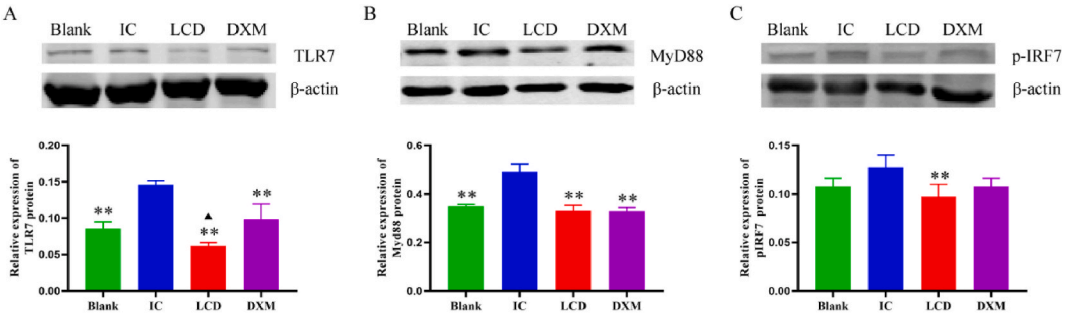

Fig. 4. The result of western blotting indicated that LCD remarkably reduced the relative expression of TLR7, MyD88, and phosphorylation of IRF7 protein in TLR7-pIRF7 pathway in DLCs. β-actin was used as control protein. A TLR7 protein expression showed in Western blotting, B MyD88 protein expression showed in Western blotting, C Phosphorylation of IRF7 protein expression showed in Western blotting. Comparison with IC group, $^{**}P < 0.01$. Comparison with Blank group, $^{\blacktriangle}P < 0.05$,$^{\blacktriangle\blacktriangle}P < 0.01$. DLC: dendritic like cell, IC: immune complex, LCD: Langchuangding, DXM: dexamethasone.

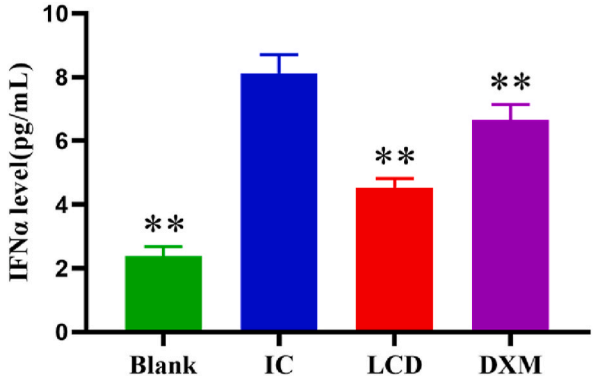

Fig. 5. The level of IFNα evaluated by ELISA in supernatant of DLCs. Comparison with IC group, $^{**}P < 0.01$. DLC: dendritic like cell, IC: immune complex, LCD: Langchuangding, DXM: dexamethasone, IFNα: interferon α.

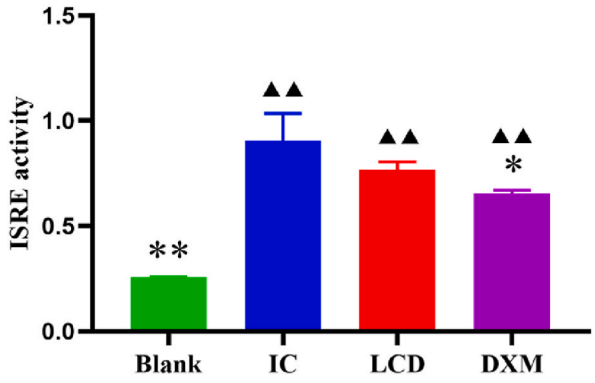

Fig. 6. ISRE activity in 293T cells. Comparison with IC group, $^{*}P < 0.05$, $^{**}P < 0.01$, Comparison with Blank group, $^{\blacktriangle\blacktriangle}P < 0.01$. IC: immune complex, LCD: Langchuangding, DXM: dexamethasone, ISRE: interferon stimulated response element.

blood was related to SLE disease activity, and peripheral blood pDC was more obviously decreased in patients with active SLE, and our previous research was also confirmed the conclusion [18]. In this study, LCD combined with western medicine and western medicine treatment increased the pDC in peripheral blood, indicating that LCD may increase the proportion of peripheral blood pDC in SLE patients.

High concentrations of circulating IFN-I and IFN-stimulating gene markers in SLE patients correlate with SLE severity [19]. IFNα is an antiviral and immunomodulatory cytokine that can induce an autoimmune response through the function of a series of downstream genes, and have shown increased expression of IFNα-induced genes in PBMC of SLE patients [20]. Increased IFNα is closely associated

with the most severe SLE manifestations, and is essential for pDC survival, activation, and migration in vivo [21–23]. In this study, the gene microarray detection showed that LCD could down-regulate the genes of IFNα related signaling pathways in PBMC cells of SLE patients. The plasma IFNα level of SLE patients was significantly increased, and therapy with LCD combined with western medicine was significantly lower than that of western medicine therapy.

The recognition of pathogens by pDC is mainly mediated by the recognition of nucleic acid, RNA and DNA of invading organisms by TLR7 and TLR9 respectively, thus participating in innate immunity and adaptive immunity [24]. pDC produce IFN-I dependent on TLR ability, and IFNα production depends on the activation of IRF-7 and its migration to the nucleus [25]. IRF-7 forms a complex with MyD88, IRAK-1, IRAK-4, and TRAF6, and IRAK-1 phosphorylates IRF-7 directly, and activate the signaling way [7,26].

In this study, murine-induced DLCs were employed to observe the effects of LCD on TLR7-IRF7-IFNα signaling pathway, and 293T cell line was introduced to observe the effects of LCD on ISRE gene. IC, as a stimulant, can activateTLR7 signaling way in DLCs. The results showed that LCD down-regulate *MyD88* and *Irf* 7 mRNA expression in DLCs, inhibit the expression of TLR7, and MyD88, inhibit the phosphorylation of IRF7 protein, and ultimately decrease IFNα. IC can activate the transcriptional activity of IRF7 in 293T cells, and LCD slightly inhibit the transcriptional activity of IRF7, indicating that LCD treatment on SLE patients may be through TLR7-IRF7-IFNα signaling pathway. This study only focused on the TLR7-IRF7-IFNα signaling pathway, and more mechanism studies are needed for multi-target of LCD in the treatment of SLE.

5. Conclusions

Our research explored the underlying mechanisms by which LCD benefit to SLE. Our data indicated that LCD treatment for SLE may be through TLR7-IRF7-IFNα signaling pathway, and IRF7 may be a promising therapeutic target for the treatment of SLE.

Ethics approval and consent to participate

This study was approved by the Medical Ethics Committee of Zhejiang Chinese Medical University (2015zjtcm-016), and obtained informed consent of the participants, and approved by the Laboratory Animal Management and Ethics Committee of the Zhejiang Chinese Medical University (IACUC-20181224-15).

Consent for publication

Not applicable.

Funding

This work was supported by the National Natural Science Foundation of China (No. 81973829, No. 81873266), the Zhejiang TCM Science and Technology Plan of China (No. 2021ZA06), Special research projects in the State Administration of Traditional Chinese Medicine (No. 201507001-4).

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Meijiao Wang: Writing – original draft, Methodology, Data curation, Conceptualization. **Yiyang Zhang:** Validation, Investigation, Data curation. **Yingqi Zhai:** Methodology, Investigation, Formal analysis. **Haichang Li:** Writing – review & editing, Methodology, Conceptualization. **Zhijun Xie:** Writing – review & editing, Project administration, Funding acquisition. **Chengping Wen:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Not applicable.

Abbreviations

SLE	systemic lupus erythematosus
LCD	Langchuangding

pDC	plasmacytoid dendritic cell
DLC	dendritic like cell
CWM	Chinese medicine combined with western medicine
WM	western medicine
SLEDAI	systemic lupus erythematosus disease activity index
dsDNA	double-stranded DNA
IC	immune complex
ISRE	interferon stimulated response element
TCM	traditional Chinese medicine
GC	glucocorticoids
BS	blank serum
MS	medicated serum
Pred	prednisone
DXM	dexamethasone
IFN I	type I interferon
IFNα	interferon α
IL-6	interleukin-6
TNFα	tumour necrosis factor α
PMA	phorbol-12-myristate-13-acetate

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26022.

References

[1] J.K. Flynn, W. Dankers, E.F. Morand, Could GILZ Be the answer to glucocorticoid toxicity in lupus? Front. Immunol. 10 (2019) 1684.
[2] C.P. Wen, et al., Study on the influence of Jieduquyuzinyin method on the quality of life of SLE patients, Chinese J. Traditional Chinese Med. 8 (2007) 1599–1602.
[3] Y.S. Fan, et al., Study on the prevention and treatment of steroid-induced osteoporosis of systemic lupus erythematosus by Jieduquyuziyin method, Chinese J. Traditional Chinese Med. 11 (2005) 28–30+5.
[4] X.C. Wang, C.P. Wen, F. Ys, Treatment of steroid dyslipidemia in systemic lupus erythematosus by Jieduquyuziyin, Zhejiang J. Integrated Traditional Chinese and Western Med. 6 (2003) 10–12.
[5] E.F. Morand, et al., Trial of anifrolumab in active systemic lupus erythematosus, N. Engl. J. Med. 382 (3) (2020) 211–221.
[6] R. Lande, M. Gilliet, Plasmacytoid dendritic cells: key players in the initiation and regulation of immune responses, Year Immunol. 1183 (2) (2010) 89–103 (1749-6632 (Electronic)).
[7] M. Gilliet, W. Cao, Y.J. Liu, Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases, Nat. Rev. Immunol. 8 (8) (2008) 594–606.
[8] J. Li, et al., Taurine metabolism aggravates the progression of lupus by promoting the function of plasmacytoid dendritic cells, Arthritis Rheumatol. 72 (12) (2020) 2106–2117.
[9] M. Petri, et al., Derivation and validation of the systemic lupus international collaborating clinics classification criteria for systemic lupus erythematosus, Arthritis Rheum. 64 (8) (2012) 2677–2686.
[10] Y.N. Gao, et al., Jiedu-Quyu-Ziyin Fang (JQZF) inhibits the proliferation and activation of B cells in MRL/lpr mice via modulating the AKT/mTOR/c-Myc signaling pathway, J. Ethnopharmacol. 315 (2023) 1872–7573 (Electronic)).
[11] F.J. Barrat, R.L. Coffman, Development of TLR inhibitors for the treatment of autoimmune diseases, Immunol. Rev. 223 (2008) 271–283, 1600-065X (Electronic).
[12] L.J. Du, et al., Jieduquyuziyin prescription promotes the efficacy of prednisone via upregulating Nrf2 in MRL/lpr kidneys, J. Ethnopharmacol. 298 (2022) 1872–7573 (Electronic)).
[13] Y. He, et al., Jieduquyuziyin prescription alleviates SLE complicated by atherosclerosis via promoting cholesterol efflux and suppressing TLR9/MyD88 activation, J. Ethnopharmacol. 309 (2023) 116283.
[14] J. Sun, et al., Effect of lang-chuang-ding decoction on DNA methylation of CD70 gene promoter in peripheral blood mononuclear cells of female patients with systemic lupus erythematosus, Chin. J. Integr. Med. 24 (5) (2018) 348–352.
[15] T.K. Means, et al., Human lupus autoantibody-DNA complexes activate DCs through cooperation of CD32 and TLR9, J. Clin. Invest. 115 (2) (2005) 407–417.
[16] L. Farkas, et al., Plasmacytoid dendritic cells (natural interferon- alpha/beta-producing cells) accumulate in cutaneous lupus erythematosus lesions, Am. J. Pathol. 159 (1) (2001) 237–243.
[17] N. Fiore, et al., Immature myeloid and plasmacytoid dendritic cells infiltrate renal tubulointerstitium in patients with lupus nephritis, Mol. Immunol. 45 (1) (2008) 259–265.
[18] M.J. Wang, et al., Effects of langchuangding on pDC content and TLR7/TLR9/MyD88 pathway in peripheral blood mononuclear cells of patients of systemic lupus erythematosus in active stage with syndrome of L.iver and kidney yin deficiency, J. Tradit. Chin. Med. 61 (13) (2020) 1169–1175.
[19] C. Soni, et al., Plasmacytoid dendritic cells and type I interferon promote extrafollicular B cell responses to extracellular self-DNA, Immunity 52 (6) (2020) 1022–+.
[20] C.E. Weckerle, et al., Network analysis of associations between serum interferon-α activity, autoantibodies, and clinical features in systemic lupus erythematosus, Arthritis Rheum. 63 (4) (2011) 1044–1053.
[21] B.W. Higgs, et al., Patients with systemic lupus erythematosus, myositis, rheumatoid arthritis and scleroderma share activation of a common type I interferon pathway, Ann. Rheum. Dis. 70 (11) (2011) 2029–2036.
[22] E.C. Baechler, et al., Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus, Proc Natl Acad Sci U S A 100 (5) (2003) 2610–2615.
[23] C. Asselin-Paturel, et al., Type I interferon dependence of plasmacytoid dendritic cell activation and migration, J. Exp. Med. 201 (7) (2005) 1157–1167.

[24] C. Guiducci, R.L. Coffman, F.J. Barrat, Signalling pathways leading to IFN-α production in human plasmacytoid dendritic cell and the possible use of agonists or antagonists of TLR7 and TLR9 in clinical indications, J. Intern. Med. 265 (1) (2009) 43–57.
[25] K. Honda, et al., IRF-7 is the master regulator of type-I interferon-dependent immune responses, Nature 434 (7034) (2005) 772–777.
[26] T. Kawai, S. Akira, TLR signaling, Semin. Immunol. 19 (1) (2007) 24–32.