Chinese herbal medicine Jia Wei Jing Xie Yin (JWJXY) ameliorates psoriasis via suppressing the Th17 cell response

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Background: Psoriasis is a chronic autoimmune disease. At present, it is very difficult to treat; however, clinical trials have shown that the traditional Chinese medicine (TCM) treatment of psoriasis has certain advantages. The Chinese herbal medicine Jia Wei Jing Xie Yin (JWJXY) has its origins in Jing Xie Yin, a medicine created by the TCM doctor Wu Jun. Previous studies have shown that JWJXY has good clinical efficacy for patients with blood-heat type psoriasis, but its mechanism is unknown.

Methods: This paper aimed to further study the therapeutic effect and mechanism of JWJXY on an imiquimod (IMQ)-induced, psoriasis-like mouse model (0.4 mL, i.g., 6 days). The histopathological skin changes were observed by hematoxylin and eosin (HE) staining, the infiltration of cluster of differentiation 11B (CD11b) and cluster of differentiation 4 (CD4) cells was observed by immunohistochemistry, lymphocyte subsets were detected by flow cytometry, T helper (Th)17 cell expression was perceived by flow cytometry, and Th17 cell-related gene expression was detected by real-time quantitative polymerase chain reaction (qPCR).

Results: JWJXY significantly reduced the skin thickness of the IMQ-induced model mouse. Compared with that in the vehicle group, the skin tissue of the mice in the JWJXY group showed significantly reduced infiltration of CD11b+ and CD4+ T cells. Flow cytometry results showed that JWJXY decreased the proportion of B220 and Th17 cells in the spleen tissue of the mice. There was no significant effect on the proportion of Th1 or regulatory T cells (Treg) cells. Compared with that in the vehicle group, the skin tissue of the mice in the JWJXY group showed significantly decreased expression of interleukin-17A (IL-17A), IL-17F, retinoic acid receptor-related orphan receptor gamma t (RORγt), IL-1β, interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α) messenger RNA (mRNA).

Conclusions: The study confirmed the therapeutic effect of JWJXY on psoriasis. Its mechanism of action might be to inhibit the Th17 cell response but not the Th1 and Treg response.

Keywords: Jia Wei Jing Xie Yin (JWJXY); psoriasis; T helper 17 cells (Th17 cells)

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Introduction

Psoriasis is a chronic autoimmune disease that affects 2–3% of the population (1,2). This disease, which is called "bai bi" in traditional Chinese medicine (TCM), is caused by a combination of genetic and environmental factors and is known in clinical practice for its easy recurrence and persistence. Psoriasis affects the sufferers' physical and mental health, and no definite treatment is currently available. The disease is characterized by distinctive skin symptoms, the most common being erythema covered with silvery layered scales. Although the etiology of psoriasis is not fully understood, recent studies have shown that pathological interactions between immune cells and keratinocytes, activated by a combination of genetic and environmental factors, drive the development and progression of psoriasis, in which T helper (Th)17 cells and effector cytokine interleukin-17A (IL-17A) play a core part (1,3). Th17 cell response can inhibit neutrophil apoptosis and induce chemotaxis by secreting inflammatory cytokines, which initiates and spreads inflammation and changes epidermal structure, resulting in the formation of psoriatic lesions (3,4). In one study, Rizzo et al. found that IL-17A knockout mice injected with recombinant mouse IL-23 showed little epidermal hyperplasia (5). Therefore, Th17 may be a critical part of the mechanism of action of psoriasis.

The Chinese herbal medicine Jia Wei Jing Xie Yin (JWJXY) has its origins in Jing Xie Yin, a medicine created by Wu Jun, an experienced TCM doctor (6-8). JWJXY has the effect of dispelling blood stasis and wind and eliminating dampness. In previous clinical studies, we found that JWJXY could significantly reduce the Psoriasis Area Severity Index (PASI) and Dermatology Life Quality Index (DLQI) scores in patients with psoriasis vulgaris of blood-heat syndrome characterized by bright red skin lesions, increasing or rapidly expanding new rashes, red or purple tongue, and rapid pulse and so on (8). Experimental studies using imiquimod (IMQ)-induced model mice have shown that a high-dose intervention of JWJXY significantly reduced the thickness of the left ear, decreased the infiltration of inflammatory cells in the left ear, and downregulated serum IL-17 expression (9). Following previous studies, the present study investigated the effect of JWJXY on Th17 cell expression in IMQ-induced mice so as to provide a foundation for the medical use of JWJXY in psoriasis treatment. We present the following article in accordance with the ARRIVE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-967/rc).

Methods

Laboratory animals

Female BALB/c mice, 7–8 weeks old and weighing 20–22 g, were provided by Chengdu Dashuo Biotechnology Co., Ltd. (certificate No. SCXK [Sichuan] 2008-24). These animals were housed in an animal barrier system (temperature: 22±1 ℃; humidity: 55%±5%; 12-hour light-dark cycle) and were used after 1 week of housing. Nutrition and water were supplied freely after disinfection. A protocol was prepared before the study without registration. The animal experiment was approved by Medical Ethics Committee of Yunnan Provincial Hospital of Traditional Chinese Medicine (No. S2020-010), in compliance with institutional guidelines for the care and use of animals.

Drugs and reagents

The composition of JWJXY (60 kg adult dose) is as follows: buffalo horn 40 g, radix rehmanniae 30 g, lithospermum 30 g, radix scutellariae 15 g, cortex dictamni 15 g, caulis spatholobi 15 g, oldenlandia diffusa 30 g, radix rubiae 10 g, radix clematidis 10 g, rhizoma atractylodis 10 g, sophora flavescens 15 g, radix rhapontiei 15 g, and tripterygium 30 g. The drug was produced at the pharmacy of Kunming Municipal Hospital of TCM. The procedure was as follows: add 10 times water, soak for 30 minutes, boil for 30 minutes, decoct 3 times, combine the decocted liquid, filter with gauze, centrifuge, evaporate with a rotary evaporator to concentrate the extract, weigh the mass, and convert 9.17 g of raw medicine into 1 g of extract of the original medicine. Based on the conventional dose of JWJXY used in the clinical treatment of adult psoriasis and the validation of previous studies, the concentration and dose of JWJXY were determined according to the conversion formula of body surface area between adult and mouse and the conversion ratio of drug dose. IMQ cream was purchased from UK 3M Health Care Limited (batch No. GTI080A, Loughborough, UK). Methotrexate tablets were purchased from Tonghua Maoxiang Pharmaceutical Co., Ltd. (batch No. 190902, Tonghua, China). APC anti-mouse IL-17A (TC11-18H10.1), fluorescein isothiocyanate (FITC) rat anti-mouse interferon gamma (IFN-γ) antibody, FITC rat anti-mouse cluster of differentiation 4 (CD4)

antibody (GK1.5), 488 anti-mouse/rat/human forkhead box P3 (FOXP3) antibody (150D), and a FOXP3/Transcription Factor Staining Buffer Set were purchased from Invitrogen (Waltham, MA, USA). Anti-cluster of differentiation 11B (CD11b) and anti-CD4 antibodies were purchased from Abcam (Cambridge, UK). Phycoerythrin (PE)/Cyanine7 anti-mouse CD4 antibody (Gk1.5), PE rat anti-mouse CD4 (H129.19), APC anti-mouse CD3 (145-2C11), and PE rat anti-mouse CD45R/B220 (RA3-6B2) were purchased from BioLegend (San Diego, CA, USA). Fetal bovine serum (FBS) was purchased from BI Israel (Tel Aviv, Israel). Dulbecco's phosphate-buffered saline (DPBS) was purchased from Gibco (USA). SYBR Premix Ex Taq II and a reverse transcription kit were purchased from Takara Biotechnology (Dalian) Co., Ltd. (Dalian, China). Trizol lysate (Buffer RZ) and an RNA extraction kit were purchased from Tiangen Biotech Co., Ltd. (Beijing, China).

IMQ-induced psoriatic BALB/c mice

Fifty female BALB/c mice, with an average body weight of 18–20 g, were randomly divided into 5 groups: a vehicle group; a low-, medium-, and high-dose JWJXY group (8, 16, and 32 g/kg, respectively); and a methotrexate-positive control group (MTX group), with 10 mice per group. All mice were housed in clean animal feeding rooms. The hair from the central area of the ear of the mouse was carefully shaved, the surface hair was removed with a mild depilatory cream, and moisturizer was applied to the surface for later use. If the hair grew out again during the test, hair removal was repeated 1–2 times. IMQ cream of 5% was evenly applied to the alopecia area at a dose of 50 mg/cm^2, once a day for 6 days. The vehicle group was administered 0.4 mL of normal saline by i.g., and the dose group was administered 0.4 mL of the corresponding concentration of the drug solution by i.g., once a day for 6 days.

Hematoxylin and eosin (HE) staining

Six days after drug administration, the mice in each group were sampled, and the samples were fixed in 4% paraformaldehyde solution. Transparent tissue sections were made by embedding, sectioning, drying, dewaxing, hydrating, and HE staining. The tissue structure and inflammatory cell infiltration of the skin of the mice in each group were observed under an inverted light microscope, and images were collected with a digital camera.

Immunohistochemistry

The sections were routinely dewaxed in water, washed with phosphate buffer saline (PBS), incubated, mixed with the primary antibody, mixed with the secondary antibody, stained, dehydrated, and then mounted to make transparent sections. The infiltration of CD11b and CD4 cells in the skin of the mice in each group was observed under an inverted optical microscope, using images that had been composed with a digital camera.

Flow cytometry

Samples were acquired using a flow cytometer (FACSCanto II, BD Biosciences, Franklin Lakes, NJ, USA), and the number and proportion of the lymphocyte subsets CD3, CD4, and B220 were analyzed using FlowJo software (BD Life Sciences). The splenocytes of the mice were harvested and blocked with rat anti-mouse CD16/CD32. Cells were fluorescently characterized and incubated with related antibodies at 4 ℃ for 15 minutes. In the presence of brefeldin A, spleen cells were restimulated with phorbol 12-myristate 13-acetate (PMA) and ionomycin for 4 hours. After incubation, cells were composed, blocked with rat anti-mouse CD16/CD32, and stained with PE/Cyanine7 anti-mouse CD4 antibody. Cells were fixed, permeated, and stained with fluorochrome-conjugated anti-mouse IL-17A and anti-mouse IFN-γ. The samples were acquired using the flow cytometer, and CD4$^+$IL-17A$^+$ cells, CD4$^+$FOX3$^+$ cells, and CD4$^+$IFN-γ$^+$ cells as a percentage of CD4$^+$ T cells were analyzed using FlowJo software.

RNA extraction and real-time quantitative PCR

Mouse skin cells were collected, and RNA was isolated using a RNeasy kit (Qiagen, Hilden, Germany). Complementary DNA (cDNA) was manufactured from 1 μg of total RNA and detected by quantitative polymerase chain reaction (q-PCR) using a SYBR Premix Ex Taq II kit. The expression of IL-17A, IL-17F, IL-1β, IL-23, tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), retinoic acid receptor-related orphan receptor gamma t (RORγt), and chemokine receptor 6 (CCR6) messenger RNA (mRNA) in the mouse skin cells was determined, and the relative expression amount of β-actin in each sample was calculated by applying the $2^{-\Delta\Delta CT}$ approach. The primer sequences of specific genes were shown in *Table 1*.

Statistical methods

Statistical analyses were performed using SPSS 20.0 software (IBM Corp., Armonk, NY, USA). Data are expressed as mean ± standard deviation (SD) of indicated experiments. A chi-square test was used to analyze the data, and a *t*-test was used to compare the 2 samples. P<0.05 was considered to be statistically significant.

Table 1 Sequences of target gene specific primer

Gene name	Primer sequences (5'-3')
Mouse *β-actin*	Forward: 5'-GGCTGTATTCCCCTCCATCG-3'
	Reverse: 5'-CCAGTTGGTAACAATGCCATGT-3'
Mouse *IL-17A*	Forward: 5'-TTTAACTCCCTTGGCGCAAAA-3'
	Reverse: 5'-CTTTCCCTCCGCATTGACAC-3'
Mouse *IL-17F*	Forward: 5'-CTGTTGATGTTGGGACTTGCC-3'
	Reverse: 5'-TCACAGTGTTATCCTCCAGG-3'
Mouse *IFN-γ*	Forward: 5'-ATGAACGCTACACACTGCATC-3'
	Reverse: 5'-CCATCCTTTTGCCAGTTCCTC-3'
Mouse *RoRγt*	Forward: 5'-TGTCCTGGGCTACCCTACTGA-3'
	Reverse: 5'-CACATTACACTGCTGGCTGC-3'
Mouse *IL-1β*	Forward: 5'-GCAACTGTTCCTGAACTCAACT-3'
	Reverse: 5'-ATCTTTTGGGGTCCGTCAACT-3'
Mouse *CCR6*	Forward: 5'-CCTGGGCAACATTATGGTGGT-3'
	Reverse: 5'-CAGAACGGTAGGGTGAGGACA-3'
Mouse *IL-23p19*	Forward: 5'-ATGCTGGATTGCAGAGCAGTA-3'
	Reverse: 5'-ACGGGGCACATTATTTTTAGTCT-3'

IL, interleukin; IFN, interferon; RoRγt, retinoic acid receptor-related orphan receptor gamma t.

Results

Efficacy of JWJXY on IMQ-induced psoriasis (ear thickness, HE staining)

IMQ is a toll-like receptor 7/8 (TLR7/8) agonist that induces psoriasis in mouse models with similar changes to human psoriasis, such as epidermal thickening, aberrant differentiation of keratinocytes, inflammatory cell infiltration, and associated inflammatory cytokines (10). It is generally accepted that lesions in this model are mediated principally via the IL-23/IL-17 axis (11). We established a mouse model of psoriasis induced via IMQ, in which normal saline, MTX (2 mg/kg), and low, medium, and high doses of JWJXY (8, 16, and 32 g/kg, respectively) were administered for 6 days. Ear thickness (*Table 2, Figure 1A*) and body weight (*Table 3, Figure 1B*) were measured on day 1, day 3, and day 5. The ear thickness of the mice reflected the severity of the rash. On the 5th day, the severity of the skin rashes in the medium dose JWJXY group was significantly lower than that in the vehicle group. Body weight in the MTX group decreased markedly and arrived on the top till 5th day. The variation in body weight in the vehicle group and the MTX control group was statistically significant. Other groups showed no significant change in body weight. Histopathological examination of the lesions revealed a significant decrease in pathological lesions in the medium and high dose JWJXY group and in the MTX group (*Figure 1C*). These results confirm that JWJXY can treat experimental psoriasis in mice.

The severity of psoriatic skin lesions in IMQ-induced mice was improved by JWJXY. A mouse model of psoriasis induced by IMQ was established. Each group was administered either a saline vehicle, MTX (2 mg/kg), or a low, medium, or high dose of JWJXY (8, 16, and

Table 2 Effect of JWJXY on the left ear thickness of IMQ-induced model mice (mean ± SD, n=10)

Group	Dose	Day 1 thickness (mm)	Day 3 thickness (mm)	Day 5 thickness (mm)
Vehicle	–	0.150±0.020	0.188±0.036	0.227±0.038
MTX	2 mg/kg	0.153±0.023	0.167±0.025	0.201±0.024
JWJXY				
Low dose	8 g/kg	0.150±0.026	0.163±0.021	0.201±0.027
Medium dose	16 g/kg	0.133±0.011	0.155±0.023	0.183±0.021*
High dose	32 g/kg	0.174±0.048	0.170±0.020	0.201±0.019

*, P<0.05 *vs.* the vehicle group. IMQ, imiquimod; JWJXY, Jia Wei Jing Xie Yin; MTX, methotrexate-positive control group; SD, standard deviation.
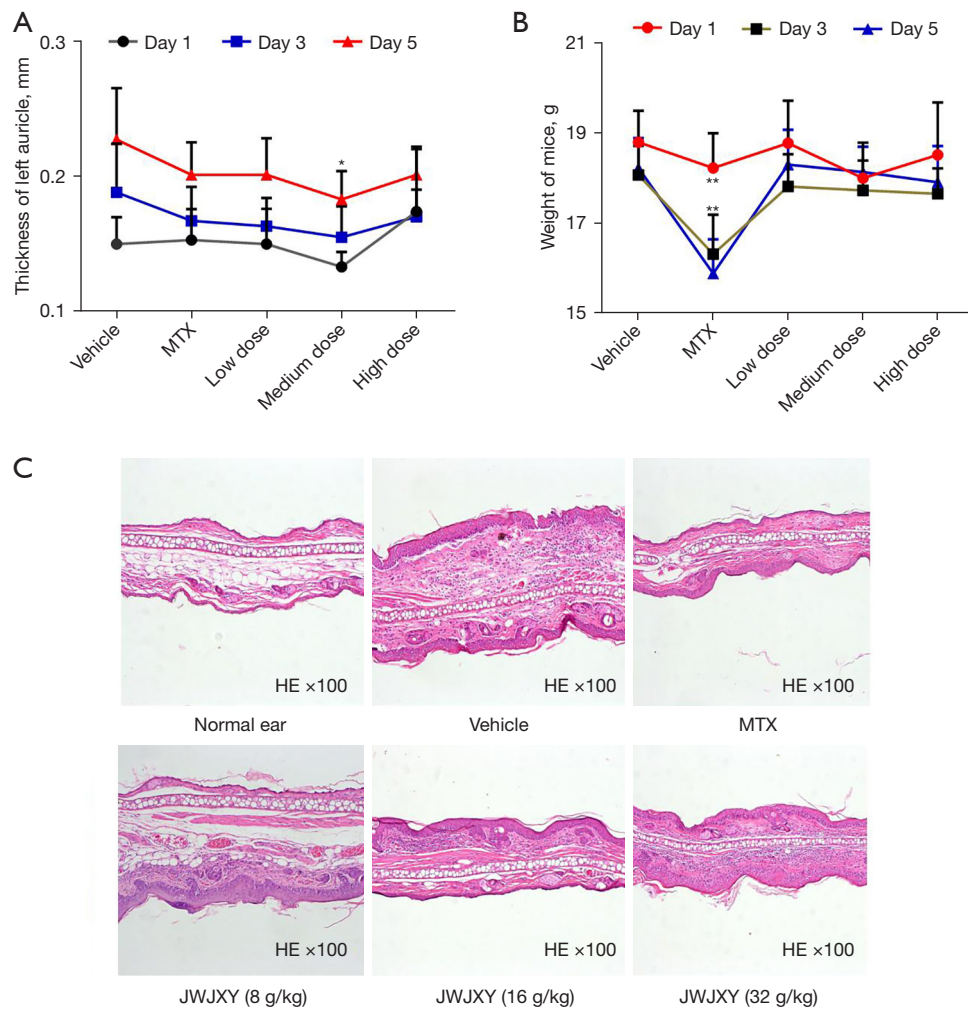
Figure 1 Effect of JWJXY on the body weight and rash severity of the mouse model. (A) Comparison of the effects of each group's intervention on the ear thickness of the mice; (B) comparison of the effects of each group's intervention on the body weight of the mice; (C) histopathological expression of the skin lesions of the mice in each group. *, P<0.05; **, P<0.01 *vs.* the vehicle group. JWJXY, Jia Wei Jing Xie Yin; MTX, methotrexate-positive control group; HE, hematoxylin and eosin.

Table 3 Effect of JWJXY on the body weight of IMQ-induced model mice (mean ± SD, n=10)

Group	Dose	Day 1 weight (g)	Day 3 weight (g)	Day 5 weight (g)
Vehicle	–	18.780±0.696	18.060±0.648	18.210±0.666
MTX	2 mg/kg	18.210±0.772	16.310±0.870**	15.878±0.758**
JWJXY				
Low dose	8 g/kg	18.760±0.944	17.800±0.720	18.290±0.770
Medium dose	16 g/kg	17.990±0.788	17.720±0.660	18.120±0.564
High dose	32 g/kg	18.500±1.160	17.640±0.564	17.900±0.800

**, P<0.01 *vs.* the vehicle group. IMQ, imiquimod; JWJXY, Jia Wei Jing Xie Yin; MTX, methotrexate-positive control group; SD, standard deviation.

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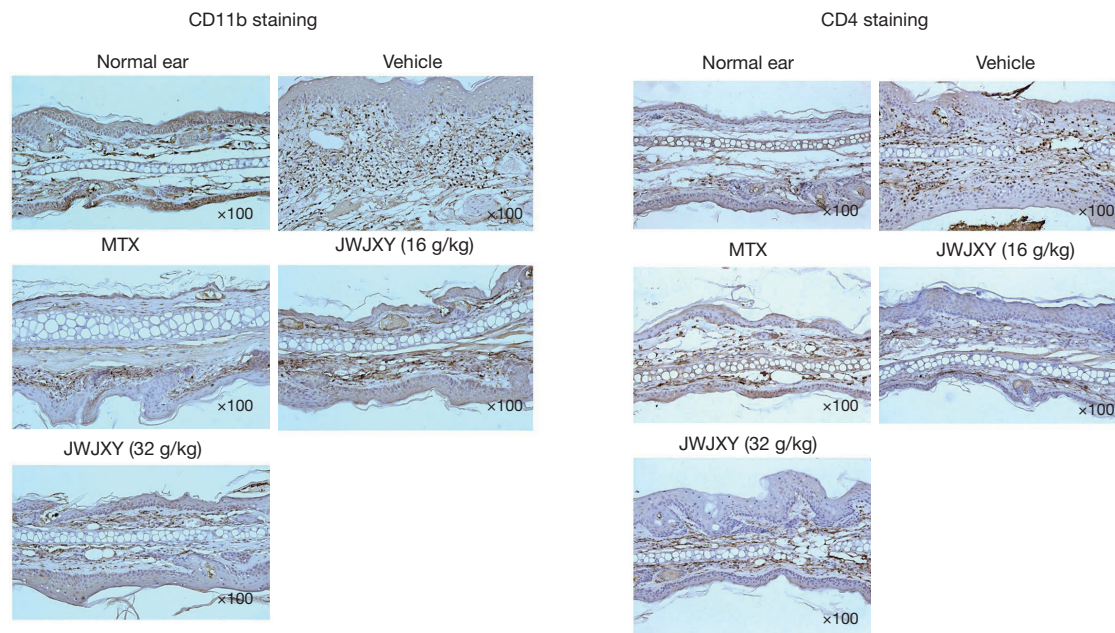Yang et al. JWJXY ameliorates psoriasis by suppressing Th17 response

Figure 2 Effect of JWJXY on CD11b and CD4 cell infiltration in psoriatic skin induced by IMQ. JWJXY can improve the infiltration of CD11b and CD4 cells in the psoriatic skin of mice induced by IMQ. A mouse model of IMQ-induced psoriasis was established. Each group was administered either a saline vehicle, MTX (2 mg/kg), or a low, medium, or high dose of JWJXY (8, 16, and 32 g/kg, respectively) for 6 days. The mice were sacrificed for immunohistochemical examination of the skin lesions. The results showed that medium and high dose of JWJXY could improve the infiltration of CD11b and CD4 cells in the psoriatic skin of mice induced by IMQ (n=10). CD, cluster of differentiation; IMQ, imiquimod; JWJXY, Jia Wei Jing Xie Yin; MTX, methotrexate-positive control group.

32 g/kg, respectively) for 6 days after the day of modeling. The ear thickness (*Figure 1A*) and body weight (*Figure 1B*) were measured on day 1, day 3, and day 5. The mice were sacrificed for histopathological examination of the skin lesions (*Figure 1C*). Results are expressed as mean ± SD (n=10). Ear thickness compared with that of the vehicle group: P<0.05. Body weight compared with that of the vehicle group: P<0.01.

Effect of JWJXY on CD11b and CD4 cell infiltration in psoriatic skin induced by IMQ

Despite the complex pathogenesis of psoriasis, recent basic and clinical studies have indicated that psoriasis is an immune-mediated inflammatory disease. Adaptive and innate immune cells play an important role in the expansion and progression of psoriasis (1). We performed an immunohistochemical examination of the skin lesions in our psoriasis mouse model, and the results showed that medium and high doses of JWJXY could ameliorate the CD11b and CD4 cell infiltration in psoriatic skin induced by IMQ. Compared with that in the vehicle group, the skin infiltration

of CD11b and CD4 cells was significantly reduced in the medium and high dose JWJXY groups (*Figure 2*).

Effect of JWJXY on IMQ-induced subsets of psoriatic lymphocytes

To investigate the effect of JWJXY on spleen cell surface markers in IMQ-induced mice, the quantity of CD3, CD4, and B220 cells within spleen subsets of the mice in the JWJXY group and the vehicle group were analyzed by flow cytometry. Surface marker staining showed a noteworthy decline in the quantity of B220 cells. There was no apparent effect on the quantity of $CD3^+$ T and $CD4^+$ T cells in spleen leukocytes (*Figure 3*).

Effect of JWJXY on Th17 cell expression in IMQ-induced psoriasis

T lymphocytes play a key role in the pathogenesis of psoriasis. Naive T cells can be differentiated into pathogenic effector cells, such as Th1 and Th17, and the immunosuppressive regulatory T cells (Tregs). To
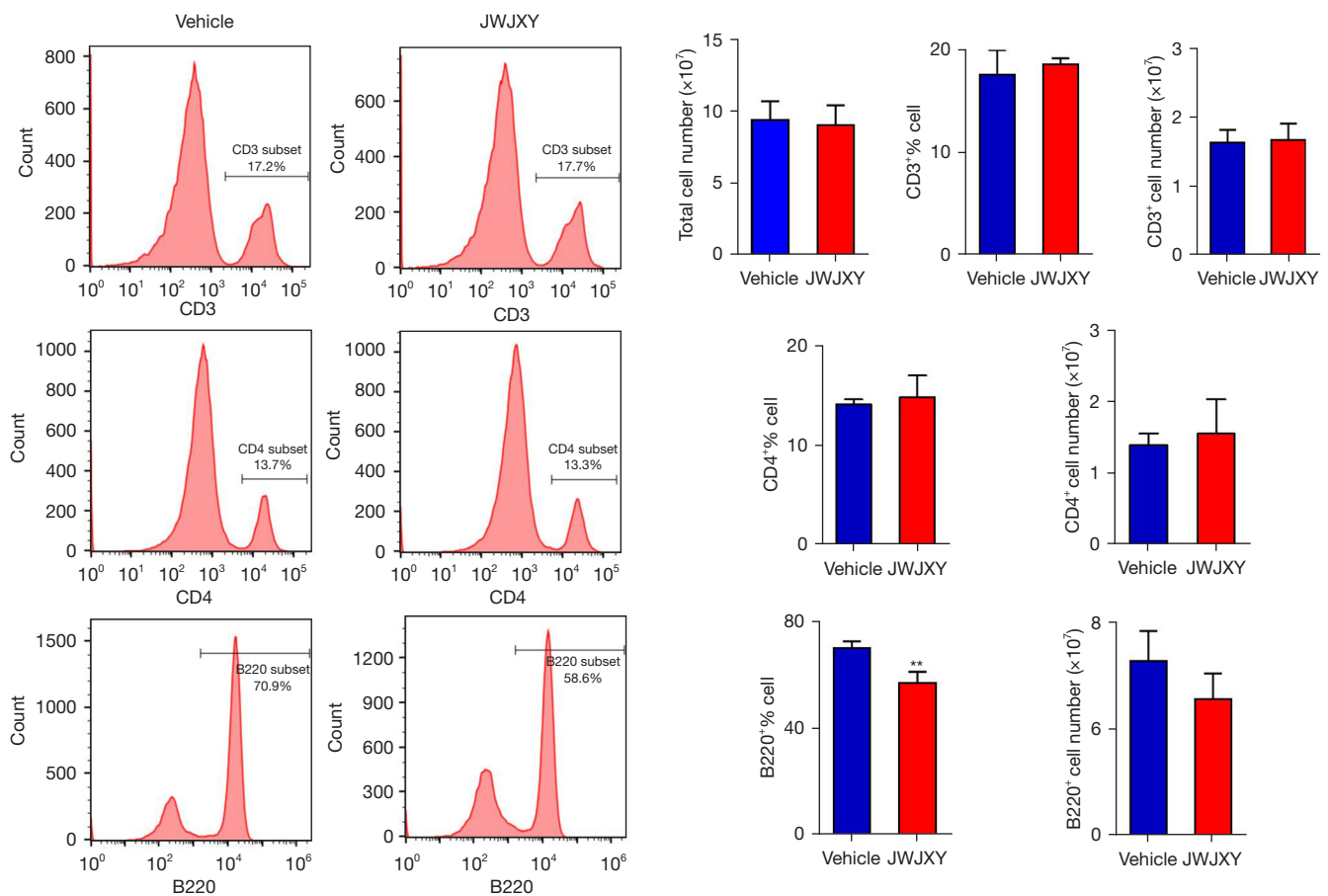
Figure 3 Effect of JWJXY on lymphocyte subsets of IMQ-induced psoriasis. JWJXY can affect the lymphocyte subsets of psoriasis induced by IMQ. Mouse splenocytes were collected and blocked by rat anti-mouse CD16/CD32, fluorescently labeled, and incubated with associated antibodies at 4 °C for 15 minutes. Samples were collected by flow cytometry and analyzed using FlowJo software. The number and proportion of CD3, CD4, and B220 cells in lymphocyte subsets were analyzed. Results are expressed as mean ± SD (n=4). **, P<0.01 *vs.* the vehicle group. CD, cluster of differentiation; IMQ, imiquimod; JWJXY, Jia Wei Jing Xie Yin; SD, standard deviation.

further clarify whether JWJXY can regulate Th cell subsets in psoriasis, flow cytometry was used to examine the response of Th17 (IL-17A$^+$), Th1 (IFN-γ$^+$), and Treg (CD4$^+$FOXP3$^+$) in psoriatic mice. The results showed that JWJXY significantly inhibited the expression of IL-17A$^+$ in CD4$^+$ T cells but did not modify the proportion of IFN-γ$^+$ or FOXP3$^+$ in CD4$^+$ T cells (*Figure 4*). Therefore, JWJXY mainly affected the expression of Th17 cells but did not affect Th1 or Treg cells.

Effect of JWJXY on Th17 cell-related gene expression in IMQ-induced psoriasis

Th17 cells are Th cell subsets that secrete IL-17 and are derived from primary CD4$^+$ T cells, IL-17 cytokines play

an important role in organ-specific immune response in a variety of physiologic functions and disorders (12). Th17 cell differentiation is regulated via RORγt, the master regulator of Th17 cells (13). CCR6 is the only receptor for the chemokine CCL20, which combines to form a CCL20/CCR6 axis that induces chemotaxis of Th17 cells and is involved in the development of psoriatic lesions (14). Our study showed that the Th17 response and IL-17A production in the psoriasis mouse model were significantly inhibited by JWJXY. To further explore the effect of JWJXY on IL-17A production, we used q-PCR to determine whether JWJXY may influence the responses of Th17-linked genes within the skin cells of mice with IMQ-induced psoriasis (*Figure 5*). The results revealed that the transcription of IL-17A, IL-17F, RORγt, TNF-α, IL-1β,
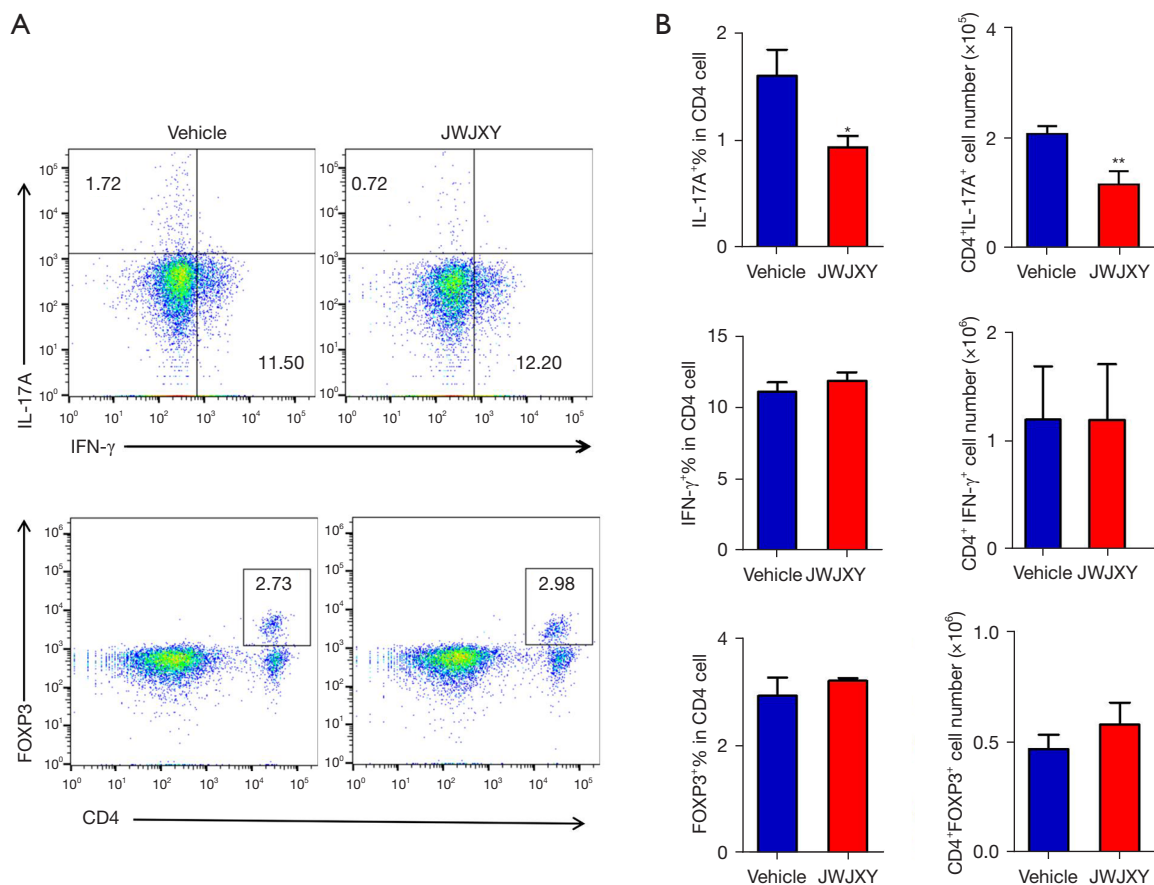
Figure 4 The expression of Th17 (IL-17A$^+$), Th1 (IFN-γ$^+$), and Treg (CD4$^+$FOXP3$^+$) in mice with IMQ-induced psoriasis was detected by flow cytometry. JWJXY treatment markedly suppressed the Th17 cell response in psoriasis-like mice induced by IMQ. Splenocytes from the model mice were restimulated for 4 hours with PMA and ionomycin in the presence of brefeldin A. After incubation, the cells were collected and blocked with rat anti-mouse CD16/CD32 and then stained with PE/Cyanine7 anti-mouse CD4 antibodies. The cells were fixed, permeabilized, and stained intracellularly with fluorochrome-conjugated anti-mouse IL-17A and anti-mouse IFN-γ. Samples were acquired using a flow cytometer and analyzed using FlowJo software. Results are expressed as mean ± SD (n=4). *, P<0.05; **, P<0.01 *vs.* the vehicle group. CD, cluster of differentiation; IFN-γ, interferon gamma; IL-17A, interleukin 17A; IMQ, imiquimod; JWJXY, Jia Wei Jing Xie Yin; PMA, phorbol 12-myristate 13-acetate; SD, standard deviation; Th17, T helper 17; Treg, regulatory T cells.

and IFN-γ was notably decreased in the JWJXY group, while the CCR6 and IL-23 response was uninfluenced.

Discussion

JWJXY has its origins in Wu Jun's Jing Xie Yin, which has been used in clinic to treat psoriasis for many years with good effect. Modern pharmacological study has shown that buffalo horn has antipyretic, sedative, anti-inflammatory, and hemostatic effects (15). Lithospermum has anti-inflammatory, antibacterial, anti-viral, anti-tumor, anti-allergic, and anti-thrombotic pharmacological effects (16).

Alkannin inhibits the proliferation of HaCaT cells mediated by IL-22 and IL-17, inhibits the secretion of inflammatory factors such as S100A7/A8 induced by IL-22 and IL-17 and chemokines such as CXCL1/2 and CCL20, and inhibits IL-17-induced vascular endothelial growth factor (VEGF) expression by blocking the Janus kinase 2/signal transducers and activators of transcription 3 (JAK2/STAT3) pathway (17-19). Oldenlandia diffusa has anti-tumor, anti-inflammatory, and antioxidant activity. In addition, it can modulate immunity and has pharmacological effects of reducing cardiac contractions (20). Tripterygium has good therapeutic effect on autoimmune diseases such as psoriasis,
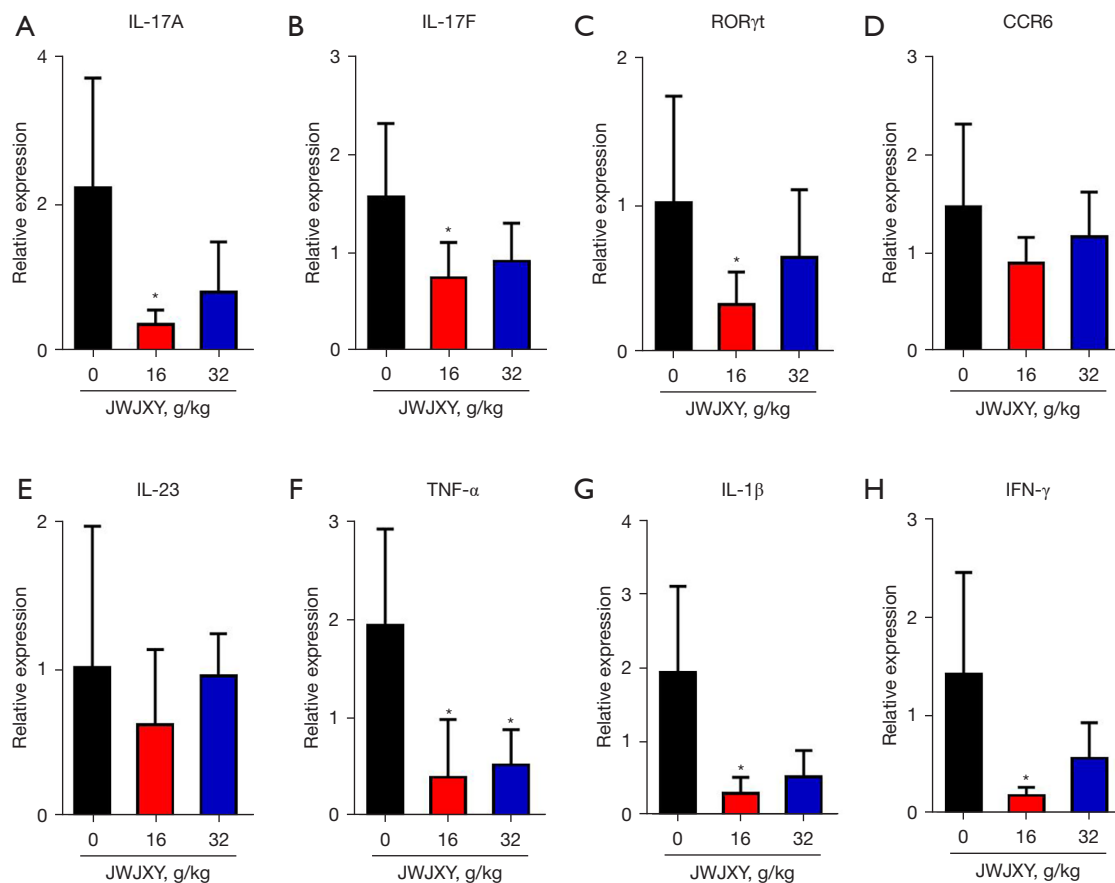
Figure 5 Effect of JWJXY on the expression of related genes in IMQ-induced psoriasis Th17 cells (IL-17A, IL-17F, RORγt, CCR6, IL-23, TNF-α, IL-1β, and IFN-γ). JWJXY markedly downregulated transcription expression in Th17-related genes. Skin from psoriasis-like mice induced by IMQ was prepared, and total RNA was isolated using RNeasy kits. Then, 1 μg of total RNA was used to synthesize cDNA. q-PCR assay was performed using a SYBR Premix Ex Taq II kit. Relative mRNA expression was calculated as the fold increase using the $2^{-\Delta\Delta CT}$ method. The housekeeping gene was β-action. Results are expressed as mean ± SD (n=6). *, P<0.05 *vs.* the vehicle group. CCR6, chemokine receptor 6; IFN-γ, interferon gamma; IL, interleukin; IMQ, imiquimod; JWJXY, Jia Wei Jing Xie Yin; q-PCR, quantitative polymerase chain reaction; RORγt, retinoic acid receptor-related orphan receptor gamma t; SD, standard deviation; Th17, T helper 17; TNF-α, tumor necrosis factor alpha; Treg, regulatory T cells.

as it can suppress inflammation, antagonize inflammatory mediators, and influence cellular and humoral immunity (21).

IMQ-induced psoriatic lesions in BALB/c mice are mediated by the IL-23/RORγt/IL-17 axis. The lesions are characterized by increased epidermal hyperplasia, aberrant distinction, neutrophilic epidermal aggregation in microabscesses, neovascularization, and infiltration with CD4⁺ T cells, CD11c⁺ dendritic cells, and serous dendritic cells. IMQ is widely used to model psoriasis, as it induces IL-23, IL-17A, and IL-17F expression and increases Th17 cells in the spleen in a similar way to the pathogenesis of psoriasis in humans (22). In previous clinical study, we found

that JWJXY had an obvious therapeutic effect on psoriasis (8). Previous experimental study has shown that JWJXY downregulated serum IL-17 expression (9). However, its specific mechanism of action needs to be elucidated.

Psoriasis has been shown to be a T-cell mediated autoimmune disease. CD4⁺ T cells can be divided into 4 subsets based on the cytokines produced and their role: Th1, Th2, Th17, and CD4⁺CD25⁺ Treg (23). Th cells are key factors in the diversity of the mammalian immune response. In adaptive immunity, Th cells clear infected cells by secreting unique cytokine subsets. For example, Th1 cells produce large amounts of IFN-γ to eliminate

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intracellular pathogens, Th17 cells produce IL-17A and IL-17F and defend the host against fungi and extracellular bacteria (24). The Th17 specific transcription factor RORγt is a Th17 marker that promotes IL-17A gene expression and induces Th17 cell differentiation (25). Th17 cells and their effector factors such as IL-17A and IL-17F mediate the progress of psoriatic lesions. The results of our animal experiment showed that JWJXY reduced the transcription of the Th17 cell-related genes IL-17A, IL-17F, RORγt, TNF-α, IL-1β, and IFN-γ in mouse spleen, which is consistent with the results of previous clinical blood tests. Immunohistochemistry showed that JWJXY decreased the infiltration of CD11b and CD4 cells in the skin of mice with IMQ-induced psoriasis. JWJXY significantly inhibited the secretion of IL-17A and the expression of IL-17A$^+$ in CD4$^+$ T cells in the specific immune response of the psoriasis model but did not affect the proportion of IFN-γ$^+$ or FOXP3$^+$ in CD4$^+$ T cells. Therefore, JWJXY represses the response of Th17 cells in psoriasis but does not influence the responses of Th1 cells or Treg cells. These results suggest that JWJXY may downregulate the expression of IL-17A via constraining the Th17-mediated inflammatory response and limiting the activation of pro-inflammatory response and neutrophils, which may be the mechanism of its therapeutic effect.

In conclusion, this study showed that JWJXY alleviates the damage of psoriasis by inhibiting the differentiation of Th17 cells, which provides an experimental basis for continued research on the clinical application of JWJXY in the treatment of psoriasis. However, this study has some limitations. The Chinese herbal compound used in our study has a complex composition, and its functional components were not studied. In addition, the specific molecular mechanism of the treatment of psoriasis was not further studied and will be the subject of our future research.

Acknowledgments

Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-967/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-967/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The animal experiment was approved by Medical Ethics Committee of Yunnan Provincial Hospital of Traditional Chinese Medicine (No. S2020-010), in compliance with institutional guidelines for the care and use of animals.

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