Effect of Xinfeng capsule on pulmonary function in a adjuvant arthritis rat model

Lei Wan, Jian Liu, Chuanbing Huang, Yuan Wang, Li Zheng

Lei Wan, Jian Liu, Chuanbing Huang, Yuan Wang, Department of Rheumatism Immunity, the First Affiliated Hospital, Anhui University of Chinese Medicine, Hefei 230031, China
Li Zheng, Department of Cancer Biology, City of Hope National Medical Center and Beckman Research Institute, California 91010, USA

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Correspondence to: Prof. Jian Liu, Department of Rheumatism Immunity, the First Affiliated Hospital, Anhui University of Chinese Medicine, Hefei 230031, China. liujianahzy@126.com
Telephone: +86-551-62838582
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Abstract

OBJECTIVE: To observe the relationship between reduced pulmonary function and regulatory T cells (Tregs) and helper T cells (Th1/Th2) drift in a rat model of adjuvant arthritis (AA), and to study the impact of Xinfeng capsule (XFC) on pulmonary function and investigate the mechanism of action.

METHODS: Forty rats were randomly divided into normal control group (NC), model control group (MC), Tripterygium glycosides tablet group (TPT), and XFC group, with 10 in each. Except for the NC group, AA was induced in all rats by intracutaneous injection of 0.1 mL Freund’s complete adjuvant in the right paw. On the 19th day after modeling, the NC and MC groups were given physiological saline (0.9%), while the TPT and XFC groups were given TPT (10 mg/kg) and XFC (2.4 g/kg), once daily, respectively. Thirty days after administration, changes in paw swelling, arthritis index (AI), pulmonary function parameters, levels of serum γ-interferon (IFN-γ) and interleukin (IL)-4, Tregs in peripheral blood, and IFN-γ, IL-4, Forkhead box transcription factor 3 (FoxP3) in lung tissue were observed by enzyme-linked immunosorbent assay, flow cytometry, polymerase chain reaction, and western blot.

RESULTS: Compared with the NC group, paw swelling, AI, IFN-γ, and Th1/Th2 were increased, and pulmonary function parameters, IL-4, FoxP3 were decreased significantly in the MC group (P<0.05 or P<0.01). Pulmonary function parameters, Treg, IL-4, FoxP3 (and mRNA) were higher, and paw swelling, AI, and IFN-γ (and mRNA) were lower in the XFC group than those in the MC group. The XFC group was also much better than the TPT group in improving pulmonary function, FoxP3 mRNA, IFN-γ, IL-4, Th1/Th2, and IL-10 (P<0.05 or P<0.01).

CONCLUSION: Xinfeng capsule can improve pulmonary function by regulating the levels of Tregs, inhibiting the activation of Th1 to Th2 cells, inducing drift, maintaining cell immune suppression, correcting the imbalance of Th1/Th2, and reducing inflammatory mediators.

Key words: Arthritis, experimental; Pulmonary
INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease with chronic panarthritis as the main clinical manifestation. It not only leads to joint deformities, but is also often accompanied by other visceral lesions. The lungs contain abundant connective tissue and are therefore most often affected, resulting in a decline of pulmonary function and lung tissue damage. Studies have found that lung diseases cause reduced pulmonary function in 60%-70% of RA patients. Early research found that rats with adjuvant arthritis have reduced pulmonary function, and pulmonary function decline is closely related to Th1 and Th2 cells and imbalanced expression of CD4+ CD25+ regulatory T cells (Tregs). Two types of CD4+ Th cells have different responses to cytokines. Interferon (IFN)-γ can induce the differentiation of Th1 cells, and inhibits Th2 cell proliferation, while interleukin-4 (IL-4) induces helper T cells (Th)2 cell differentiation. There is most likely inhomogeneity within Th1 and Th2 subsets from the point of performance and effect. This is known as "cloning drift" in which one subgroup of Th1/Th2 would enhance, while another subgroup of Th1/Th2 would abate function. The balance between Th1 and Th2 cells is broken in the pathogenesis of RA and the subsets drift in the wrong directions. This may lead to autoimmune response, directly causing the deterioration of RA. It is a trend in the treatment of many diseases to adjust the drift in right direction. Studies have found that Traditional Chinese Medicine Xinfeng capsule (XFC), which consists of Huangqi (Radix Astragali Mongolicis), Yi yiren (Semen Coicis), and Wugong (Scolopendria), can not only improve symptoms and signs of joints in patients with RA, but also obviously improve their pulmonary function. XFC can improve RA symptoms such as joint swelling, joint pain and morning stiffness, and lower the indexes of joint pain and joint tenderness, reduce blood sedimentation, C-reactive protein, anti-cyclic citrullinated peptide (CCP), and rheumatoid factor. XFC can obviously improve the pulmonary function in patients with RA parameters such as forced vital capacity (VC), forced expiratory volume in 1 s (FEV1), forced respiration rate of 1 s (FEV1/FVC), forced vital capacity 25% of maximum expiratory flow (FEF25), forced vital capacity 50% of maximum expiratory flow (FEF50), forced vital capacity 75% of maximum expiratory flow (FEF75), largest tidal midexpiratory flow (MMF), and maximum expiratory flow (PEF). XFC can improve pulmonary symptoms and signs such as cough, sputum, chest tightness, wheezing, and short of breath. In this paper, the effects of XFC on Th1/Th2 cells and Tregs in rats with adjuvant arthritis (AA) were observed based on previous studies. We aimed to study the impact of XFC on pulmonary function and investigate its mechanism of action.

MATERIALS AND METHODS

Ethical approval of the study protocol

The study protocol was approved by the Ethics Committee of First Affiliated Hospital, Anhui University of Chinese Medicine (Hefei, China).

Experimental animals

Forty male Sprague-Dawley (SD) rats (180–200) g were purchased from the Experimental Animal Center of Nanjing Medical University (Nanjing, China). All animals were housed in specific pathogen-free (SPF) conditions, and had free access to water and standard rat feed.

Drugs and reagents

XFC, 0.4 g/tablet, was provided by the First Affiliated Hospital of Anhui Medical Center, (Hefei, China, batch number: 20110129). Tripterygium glycosides tablet (TPT), 10 mg/tablet, was from Hongqi Pharmaceutical Factory, Shanghai Medical University (Shanghai, China, batch number: 20101112). Freund’s complete adjuvant (FCA) was produced by Sigma-Aldrich Co., (St. Louis, MO, USA, batch number: 108k8729). IFN-γ and IL-4 assay kits were purchased from R&D Systems, Inc. (Emeryville, CA, USA, batch number: 2011021321, 2011072003). CD4 mAb (lot: 11-0040-81), CD25 (lot: 11-0040-45), and FoxP3 mAb (lot: 11-0040-76) were purchased from eBioscience, Inc. (San Diego, CA, USA). FoxP3 antibody was purchased from Santa Cruz Biotechnology, Ltd. (Santa Cruz, CA, USA, SC-28705). IFN-γ and IL-4 antibodies were purchased from Bioworld Technology, Inc. (Louis Park, MN, USA, BS3486, BS3501).

Instruments and equipment

PCR instrument was provided by Biometra Company Biometra GmbH (T-Gradient, Goettingen, Germany), and Applied Biosystems, Inc. (ABI7500, Foster City, CA, USA). Electrophoresis was from Amersham Biosciences (EPS-301, Uppsala, Sweden). Gel image analyzer was from Bio-RAD Corporation (Gel Doc XR, Hercules, CA, USA). Slicer was provided by LEICA Microsystems GmbH (KMM135, Wetzlar, Germany). Light microscopy was provided by LEICA Microsystems GmbH (DMLB, Wetzlar, Germany). Automatic microscope camera system was provided by OLYMPUS Corporation (CX31-32C02, Tokyo, Japan). Desktop high-speed refrigerated centrifuge was provided by Eppendorf AG (Centrifuge 5417R, Hamburg, Germany). Flow cytometry was provided by Beckman Coulter Inc. (Epics XL, Brea, CA, USA). A lung function analysis system was provided by Beijing Bestlab High-Tech Co. Ltd. (AniRes2003, Beijing, China).
Model replication and administration
Forty male SD rats were divided randomly into five groups: normal control (NC) group, model control (MC) group, Xinfeng capsule (XFC) group, and Tripterygium glycosides tablet (TPT) group, with 10 in each. Each group had no significant difference in baseline such as body weight, mice age, etc. (P>0.05). Except for the NC group, all others were intratracheally injected with 0.1 mL FCA into the right hindlimb. Administration began on the nineteenth day. The NC and MC groups were given 0.9% (physiological) saline (1 mL/100 g per day). The TPT and XFC groups were given TPT (10 mg/kg) and XFC (2.4 g/kg), respectively, for thirty days.

Calculation of paw swelling (E) and arthritis index (AI)
Paw volume was measured every three days. Paw swelling (E) was calculated using the following formula.11
\[ E(\%) = \frac{(V_t - V_n)}{V_n} \times 100\% \]
Where Vn and Vt represent the volume of the paw before and after modeling, respectively. Systemic and joint disease caused by inflammation was recorded every three days. The AI was classified using a five-scale method: 0 point: no swelling; 1 point: swelling on the joint of the little toe; 2 points: swelling on the metatarsal phalange joint and foot; 3 points: swelling on the hindpaw below ankle swelling; 4 points: swelling on the metatarsal phalange joint and foot; 5 points: swelling on the joint of the little toe; 6 points: swelling on the joint of the little toe and foot; 7 points: swelling on the metatarsal phalange joint; 8 points: swelling on the joint of the little toe; 9 points: swelling on the joint of the little toe and foot; 10 points: swelling on the metatarsal phalange joint and foot; 11 points: swelling on the hindpaw below ankle swelling; 12 points: swelling on the hindpaw and including ankle swelling. The sum of points for each rat was calculated, and the highest possible score was 12 points.12

Evaluation of pulmonary function
Several parameters of pulmonary function were evaluated. These included forced vital capacity (FVC) and average expiratory flow, which was calculated by dividing FVC by the value for forced expiratory flow in one second (FEV₁) and multiplying by 100%. Further, 25%, 50%, and 75% of the vital capacity of the peak expiratory flow (PEF₂, PEF₅₀, and PEF₇₅) were calculated. Calculation of the peak expiratory flow was also assessed. These measurements were obtained using the pulmonary function test apparatus for small animals thirty days after administration. Rats were anesthetized with 10% chloral hydrate (0.35 mL/100 g, i.p.). Tracheotomy and endotracheal intubation were then carried out. The rats were then put into an airtight box. The ventilator tube was connected to the mechanical ventilation apparatus to collect pulmonary function. In this setup, external pressure caused deep inspiration. Computer software was used to measure each indicator automatically.

Detection of IFN-γ and IL-4 in serum using ELISA
Detection was performed in accordance with the instructions of the enzyme-linked immunosorbent assay (ELISA) kit. The capture antibody (IL-4, IFN-γ) was diluted (1-10 μg/mL) in coating solution. One hundred μL diluted antibody was added to appropriate wells and incubated for 2 h at room temperature. Coating buffer was emptied and residual liquid removed. Plates were washed twice with 300 μL 0.05% tween-20, and then 300 μL blocking solution was added to each well and they were then incubated for 1 h at room temperature. Following this the plates were emptied and residual liquid removed. They were then washed twice with 300 μL wash solution. One hundred μL of diluted biotinylated detection antibody was added to each well and the plates were then incubated for 1 h at 37°C. Plates were then emptied and residual liquid removed. Each well was filled with wash solution, and plates were inverted to empty and tapped to remove residual liquid. This was repeated three times. One hundred μL diluted AKP conjugated streptavidin was then added to each well and incubated for 1 h at room temperature. Plates were then washed three times and given a final 5 min soak with wash solution. Each well was filled with wash solution, and repeated five times. Two hundred μL of substrate was then placed into each well. Color was developed for 30 min at room temperature. 0.05 mL 2 M of H₂SO₄ was added to each well, and plate was immediately with a plate reader at 405-410 nm.

Detection of CD4+ CD25+ FoxP3+ Treg in peripheral blood by flow cytometry
Fresh blood samples were collected from the rats, and whole blood samples were put into K3-EDTA containing tubes. Then, anti-mouse CD4-Per CP (20 µL) and anti-mouse CD25-FITC (10 µL) were added to each tube of blood (10⁶ cells per tube), respectively. Tubes were kept in dark for 15-20 min at room temperature (20°C-25°C). We then added 1 mL RBC lysate to the tube and incubated for 15-25 min in darkness. The tube contents were then washed with phosphate buffer (PBS) twice, centrifugation at 376 g, and the supernatant was discarded. 3 mL FoxP3 Buffer A were added at room temperature and incubated in darkness for 10-15 min. To each tube, we added 1 mL FoxP3 Buffer C, and mixed at room temperature in darkness for 20-30 min. FoxP3-PE (20 µL) was then added to each tube and kept in darkness for 15-20 min at room temperature. Each tube was washed with 3 mL PBS, low speed centrifugation at 211 x-g, and heavy suspension cells with 500 µL PBS buffer. The expressions of CD4+ CD25+ Tregs and CD4+ CD25+FoxP3+ Tregs were measured by flow cytometry.

Detection of IFN-γ, IL-4, and FoxP3 mRNA in lung tissue by real-time quantitative Polymerase chain reaction (PCR)
Total lung RNA was extracted, and was dissolved by adding 30 μL RNase-free ddH₂O. 500 ng of total RNA was reverse transcribed in 10 μL of reaction system (5x prime script buffer 2 μL, Prime Script RT Enzyme Mix 1 0.5 μL, Oligo dT Primer 0.5 μL, Random 6 mers 0.5 μL, diethylyrocarbonate (DEPC) wa-
Detection of IFN-γ, IL-4, and FoxP3 protein in lung tissue by western blot

Total protein in the lung tissue was extracted with Preparation of Modified Radioimmunoprecipitation (RIPA) buffer. Protein assay kit was used for concentration measurement and protein concentration was diluted to 2 μg/μL. 16 μL of protein was mixed with 4 μL of sample buffer (5×) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (10% separating gel, 5% concentrated gel). Electrophoresis was run at a constant voltage of 60 V until the top gel separated, and then it was run at a constant voltage of 100 V to separate the bottom gel. Protein was transferred to a nitrocellulose membrane with a constant current of 40 mA for 50 min. After blocking with 5% skim milk powder for 2 h, 1: 500 diluted IFN-γ, IL-4, and FoxP3 monoclonal antibody were added in, with bed shaking for 2 h. Membrane was washed in PBS three times, for 10 min each. Then, 1: 1000 diluted sheep-Horseradish Peroxidase anti mouse IgG was added in with shaking for 1 h, and washed in PBS three times, for 10 min each time. Electrochemiluminescence (ECL) reagent was used for developing the stained band, and the result was scanned with a scanner. The stained bands were analyzed with BANDSCAN software to calculate the grey value, with grey levels of stained bands were analyzed with BANDSCAN software.

RESULTS

Effects of XFC on pulmonary function in rats

Compared with the NC group, parameters of pulmonary function (FEV1, FEF50, FEF75, and PEF) were significantly reduced in the MC group. Values of FEV1, FEF50, and PEF in the XFC groups were higher than those in the MC group. Values of FEV1, FEF50, FEF75, and PEF in the XFC groups were higher than those in the MC group. Compared with the TPT group, FEV1 was increased in the XFC group (P<0.05 or P<0.01) (Table 2).

Effects of XFC on paw swelling and AI in rats

At 6 h after inducing inflammation, the right rear toes of rats in modeling groups began to swell. On the third day after inducing inflammation, paw swelling reached a peak and then decreased gradually. Some of the foretoes began to show redness and swelling on day four and five. On day 12, the AI reached a peak, and then declined gradually. Thirty days after inducing inflammation, compared with the MC group, paw swelling and AI decreased in the XFC and TPT groups (P<0.01), and no significant difference between the XFC group and TPT group was seen (P>0.05) (Table 3).

Influence of Xinfeng capsule on serum levels of IFN-γ, IL-4, and Th1/Th2 cells in rats

Compared with those in the NC group, IFN-γ and Th1/Th2 serum levels in the MC group were in-

Table 1 Primer and probe sets used for real-time quantitative PCR of lung tissue

<table>
<thead>
<tr>
<th>Gene</th>
<th>Direction</th>
<th>Sequences (Forward/Reverse 5'→3')</th>
<th>Accession No.</th>
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</thead>
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<tr>
<td>FoxP3</td>
<td>Primer</td>
<td>TCACCTGCTTTTCTTGCTATGCCT</td>
<td>NM_001108250</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AGTGCTGCTAGTGCGCCCCTGCC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>AAGCACCGCTGAGCACC</td>
<td></td>
</tr>
<tr>
<td>IL-4</td>
<td>Primer</td>
<td>TCTTGAGGCTGACCTGCTTGTCG</td>
<td>NM_201270</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TATTTCTCGTCTAGGATGCTT</td>
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<td></td>
<td>Probe</td>
<td>TCTCAAGTCTACGTGTCGTA</td>
<td></td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Primer</td>
<td>GAGAAACCCCAAGATCCAGACACA</td>
<td>NM_138880</td>
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<tr>
<td></td>
<td></td>
<td>CAGAATGCAGACCAAGACAGCTTTC</td>
<td></td>
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<td></td>
<td>Probe</td>
<td>AAAGGACCAAGATGCTACAC</td>
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<tr>
<td>β-actin</td>
<td>Primer</td>
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<td>EF156276</td>
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<tr>
<td></td>
<td></td>
<td>TGCGGGAGCGGGGTGC</td>
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<tr>
<td></td>
<td>Probe</td>
<td>ATATCGCTGCGCTCCTGT</td>
<td></td>
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</tbody>
</table>

Notes: PCR: polymerase chain reaction; FoxP3: forkhead Box P3; IL-4: interleukin-4; IFN-γ: levels of serum γ-interferon.
creased, and IL-4 was decreased (P<0.01). Compared with those in the MC group, IFN-γ and Th1/Th2 in the treatment (XFC and TPT) groups were reduced, and IL-4 was increased (P<0.05 or P<0.01). Compared with the XFC group, Th1/Th2 in TPT group was increased, and IL-4 was reduced (P<0.05). (Figure 1, 2)

Influence of Xinfeng capsule on peripheral blood level of CD4+ CD25+ Tregs in rats
CD4+ CD25+ Tregs and CD4+ CD25+ FoxP3+ Treg in peripheral blood were detected with flow cytometry. Expression of CD4+ CD25+ Tregs and CD4+ CD25+ FoxP3+ Tregs in the MC group was significantly lower than that in the NC group (Figure 3A, B, E, F). After intervention with TPT and XFC, the expression of CD4+ CD25+ Tregs and CD4+ CD25+ FoxP3+ Tregs in the peripheral blood were elevated compared with that in the MC group (P<0.05, Figure 3C, D, G, H).

Table 2 Effects of XFC on pulmonary function in rats (mL/s, x±s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>FEV₁</th>
<th>FEF₂₅</th>
<th>FEF₅₀</th>
<th>FEF₇₅</th>
<th>PEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>10</td>
<td>70±13</td>
<td>48±16</td>
<td>45±18</td>
<td>39±12</td>
<td>46±15</td>
</tr>
<tr>
<td>MC</td>
<td>10</td>
<td>50±15</td>
<td>43±14</td>
<td>30±17</td>
<td>22±15</td>
<td>31±12</td>
</tr>
<tr>
<td>TPT</td>
<td>10</td>
<td>65±20</td>
<td>44±11</td>
<td>38±17</td>
<td>29±14</td>
<td>40±16</td>
</tr>
<tr>
<td>XFC</td>
<td>10</td>
<td>66±7</td>
<td>42±19</td>
<td>35±16</td>
<td>36±17</td>
<td>37±13</td>
</tr>
</tbody>
</table>

Notes: the NC and MC groups treated with 0.9% (physiological) saline (1 mL/100 g per day). The TPT groups treated with Tripterygium glycosides tablet (10 mg/kg), the XFC groups treated with Xinfeng capsule (2.4 g/kg), for thirty days. Compared with the NC group, a P<0.01, b P<0.05; compared with the MC, c P<0.01, d P<0.05; compared with the XFC group, e P<0.05. NC: normal control; MC: model control; TPT: Tripterygium glycosides; XFC: Xinfeng capsules; FEV₁: forced expiratory flow in one second; FEF₂₅: forced vital capacity 25% of maximum expiratory flow; FEF₇₅: forced vital capacity 75% of maximum expiratory flow; PEF: peak expiratory flow.

Table 3 Effects of XFC on paw swelling, and arthritis index (x±s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Before inflammation (Paw swelling (%))</th>
<th>After administration (Paw swelling (%))</th>
<th>12 d after inflammation (Arthritis index (points))</th>
<th>After administration (Arthritis index (points))</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>10</td>
<td>1.62±0.46</td>
<td>9.87±3.45</td>
<td>0.18±0.09</td>
<td>0.32±0.13</td>
</tr>
<tr>
<td>MC</td>
<td>10</td>
<td>1.57±0.52</td>
<td>20.14±8.46</td>
<td>1.19±0.94</td>
<td>8.12±2.15</td>
</tr>
<tr>
<td>TPT</td>
<td>10</td>
<td>1.49±0.33</td>
<td>11.52±9.73</td>
<td>1.14±0.86</td>
<td>5.06±3.06</td>
</tr>
<tr>
<td>XFC</td>
<td>10</td>
<td>1.52±0.86</td>
<td>15.22±8.89</td>
<td>1.21±0.87</td>
<td>6.31±2.85</td>
</tr>
</tbody>
</table>

Notes: the NC and MC groups treated with 0.9% (physiological) saline (1 mL/100 g per day). The TPT groups treated with Tripterygium glycosides tablet (10 mg/kg), the XFC groups treated with Xinfeng capsule (2.4 g/kg), for thirty days. NC: normal control; MC: model control; TPT: tripterygium glycosides; XFC: Xinfeng capsules. Compared with the NC group, a P<0.01; compared with the MC group, b P<0.01, c P<0.05.

Influence of Xinfeng capsule on levels of IFN-γ, IL-4, and FoxP3 mRNA in lung tissue in rats
IFN-γ mRNA in lung tissue was higher, but IL-4 and FoxP3 mRNA were lower in the MC group compared with the NC group after Xinfeng capsule treatment (Figure 4).
Figure 3 Expression of regulatory T cells in peripheral blood in rats
3A-3D: Expression level of CD4+ CD25+ Treg. A: NC group, B: MC group, C: TPT group, D: XFC group; 3E-3H: Expression level of CD4+ CD25+ FoxP3+ Treg. E: NC group, F: MC group, G: TPT group, H: XFC group. NC: normal control; MC: model control; TPT: tripterygium glycosides; XFC: Xinfeng capsules. The NC and MC groups treated with 0.9% (physiological) saline (1 mL/100 g per day). The TPT groups treated with Tripterygium glycosides tablet (10 mg/kg), the XFC groups treated with Xinfeng capsule (2.4 g/kg), for thirty days.
FoxP3 mRNA were lower in the NC group than those in the MC group. The expressions of IL-4 and FoxP3 mRNA were higher, and of IFN-γ mRNA was lower in the MC group than those in the XFC group. Compared with the XFC group, FoxP3 mRNA expression was decreased in the TPT group ($P<0.05$ or $P<0.01$) (Figure 4-6).

**Influence of Xinfeng capsule on levels of IFN-γ, IL-4, and FoxP3 protein in lung tissue in rats**

The level of IFN-γ was higher, but the expression of IL-4 and FoxP3 protein were lower in the NC group than those in the MC group. Compared with the MC group, the level of IFN-γ was lower in the XFC group, while the expression IL-4 and FoxP3 were higher. Compared with the XFC group, the expressions of IL-10 and FoxP3 were lower in the TPT group ($P<0.05$ or $P<0.01$) (Figure 7).

**DISCUSSION**

Mosmann et al. showed that CD4 positive cells in mice are an inhomogeneous subgroup by application of Th cell clone culture technology and cytokines. The subgroup can be classified into two independent subsets with different functions, Th1 and Th2. Th1 and Th2 cells are formed from different evolutionary cell lines. Th1 cells are called inflammatory T cells and mainly secrete IFN-γ, IL-1, IL-2, and TNF-α, and mediate immune response associated with cytotoxic and local inflammation. Th1 cells are involved in cellular immunity and inflammation of delayed-type hypersensitivity. Th2 cells secrete IL-4, IL-5, IL-6, IL-9, IL-10, IL-11, and IL-13, and mainly stimulate B cell prolifera-
tion and antibody production. They are also associated with humoral immunity. In the present study, the typical cytokines of Th1 and Th2, IFN-γ and IL-4 were observed. IFN-γ promotes cell inflammation and is an important regulatory factor on the body’s inflammatory response and immune response. IFN-γ can also cause swelling in the joints in early RA, and stimulate synovial cells and cartilage cells to produce PGE2 and collagenase, which leads to local synovial inflammation and joint cartilage damage. It plays a central role in cytokine network of RA. IL-4 is an anti-inflammatory factor mainly secreted by Th2 cells. It can inhibit the T cells, eosinophils, NK cells, B cells and mast cells, which can produce proinflammatory cytokines and chemotactic factors. By inhibiting antigen-presenting cells, inducing T cells not to respond, and inhibiting inflammatory cells directly within the airway, IL-4 can control lung lesions in RA. Tregs can inhibit the expression of Th1 and Th2 cells, and the inhibition may be greater on Th1 cells than on the Th2 cells. Thus transformation of the immune response of polarization is regulated.

Indexes such as paw swelling degree, arthritis index, IFN-γ, and Th1/Th2 were increased in AA rats after FCA induced inflammation. At the same time, pulmonary function parameters, such as FEV1, FEF25, FEF50, PEF, and IL-4 were reduced. This indicates that immune complexes deposit in the joints in AA rats after inflammation, and further stimulate the release of inflammatory mediators. These processes involved in the local inflammatory response, prompt lesions in the lung tissue, and reduce pulmonary function. Among the pulmonary function parameters, FEF25, FEF50, and FEF50 reflect the function of small airways, and FEV1, MMF, and PEF reflect ventilation function. Therefore, small airway lesions and ventilation dysfunction are the main presentation of declining pulmonary function in AA rats. We found that CD4+ CD25+ Tregs and CD4+ CD25+ FoxP3+ Tregs, further promoting antimicrobial inflammation cytokine secretion of IL-4, and inhibiting the expression of IFN-γ. Xinfeng capsule tends to balance the Th1/Th2 cell ratio, reduce the inflammatory response and immune complex target damage of lung tissues and organs, and improve the level of pulmonary symptoms and pulmonary function. We found that IFN-γ mRNA and IFN-γ protein levels in lung tissue were decreased, using quantitative PCR and western blotting. FoxP3 (Treg surface markers), and IL-4 mRNA and protein in lung tissue were higher after the intervention of Xinfeng capsule in AA rats. This suggests that there is a balance between Th1/Th2 cells and the regulatory T cells. With increasing expression of regulatory T cells, normal immune tolerance is maintained, and the expression of Th1/Th2 cells keep balance. At the same time, because of this balance, inflammatory cell levels decrease, blood vessel osmotic pressure of lung tissue decreases, the inflammatory response to tissue organ stimulation is reduced, the ventilation function of lung tissue returns to normal, and the level of pulmonary function is increased.

Xinfeng capsule consists of Huangqi (Radix Astragali Mongolici), Yiyiren (Semen Coicis), Wugong (Scolopendra), and Leigongteng (Radix et Rhizoma Tripterygi). It is believed in Traditional Chinese Medicine, that Huangqi (Radix Astragali Mongolici) benefits Qi for consolidation of superficies, induces diuresis for removing edema, invigorates spleen for removing dampness, and dredges channel blockades. Research in recent years shows that astragalosides, the total glycosides of Radix Astragali, have good anti-inflammatory and immune regulatory effects. They can promote phagocytosis of macrophages. Astragalus polysaccharides can reduce edema and albumin leakage in acid lung injury, improve hypoxia, enhance oxidation resistance, reduce lung injury degree, ameliorate alveolitis and pulmonary fibrosis in lung tissue. Yiyiren (Semen Coicis) can not only invigorate spleen to elim-
nate dampness, but also dredge channel blockades by calming endogenous wind. Yiyiren (Semen Coicis) polysaccharide can significantly improve the percentage and phagocytosis index of peritoneal macrophages in mice with insufficient immunity, promote hemolysin and plague forming cell, and help lymphocyte transformation. It can also down-regulate the expression level of Cox-2 gene. Small doses of Yiyiren (Semen Coicis) oil (palmitic acid and palmitate as the key components) showed a respiratory stimulating effect, but large doses showed respiratory depression. It also can expand the pulmonary blood vessels to relieve cough and to improve the pulmonary function.\textsuperscript{27-29} In Chinese medicine, centipede is used to relieve spasms by calming endogenous wind, improving rigidity of muscles, activating collaterals to stop pain, and removing toxicity for eliminating stagnation. Studies show that it can improve microcirculation, prolong coagulation time, and reduce the blood viscosity. It can also lower the serum level of IL-2 and increase the levels of IL-4 and IL-10 in arthritis models, presenting an obvious anti-inflammatory analgesic effect. In clinical observation, centipede showed a strong effect in eliminating phlegm and relieving cough, especially in chronic cough.\textsuperscript{30,31} Leigongteng (Radix et Rhizoma Tripterygii) can dispel pathogenic wind and eliminate phlegm, promote blood circulation for removing obstruction in collaterals, and relieve swelling and pain. Studies confirm that triptolide, one of the main active components in Leigongteng (Radix et Rhizoma Tripterygii), can relieve pulmonary alveolitis and inhibit fibrosis in a mouse model of pulmonary fibrosis. Triptolide also showed anti-inflammatory activity in cultured lung cells in vivo in an acute lung injury model. With functions as an anti-inflammatory agent, triptolide showed strong activity in eliminating phlegm and relieving cough, especially in chronic cough.\textsuperscript{32,33}

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