Correlation analysis of Treg/Th17 cells and related cytokines in patients with psoriasis vulgaris

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Abstract

OBJECTIVE: In this research we investigate whether the circulating levels of T regulatory (Treg)/T helper 17(Th17) cells and the related inflammatory cytokines along with specific transcription factors are different between the healthy control group and the psoriatic patient groups, with three types of traditional Chinese medicine(TCM) syndromes: blood-heat syndrome(BHS),blood-stasis syndrome (BSS),blood-dryness syndrome (BDS).

METHODS: We implement the use of TCM by quantifying. A total of 67 psoriatic patients were recruited and assigned to 3 groups according to their TCM syndromes(BHS: 40 patients; BSS:14 patients and BDS: 13patients ), 21 healthy individuals were enrolled into the control group. The circulating levels of Treg/Th17 cells were assessed by flow cytometry, the levels of inflammatory cytokines of interleukin(IL)-10,tumor necrosis factor(TNF), were assessed by ELISA, and the expression of specific transcription factors of retinoic acid-related orphan receptor γt(RORγt), forkhead box P3 (Foxp3) were assessed by RT-PCR.

RESULTS: The ratio of the Th17 cells and the levels of TNF-γ RORγt were all significantly higher in the BHS and BSS groups than that of the control group (P < 0.05); the ratio of the Treg cells and the levels of IL-10,Foxp3 in the BHS group were apparently lower than that of the control group (P <0.05), whereas that of patients with BSS did not show significant difference from the control group (P > 0.05). The ratio of the Th17 cells and the levels of TNF-RORγt in the BDS group showed no significant difference from the control group(p>0.05),however, the ratio of the Treg cells, and the levels of IL-10,Foxp3 were both lower than the healthy group (p<0.05).

CONCLUSION: Based on the ratio of the Treg/Th17 cells and the levels of related cytokines in the patients’ peripheral blood in the three TCM syndromes, we determined that The ratio of the Th17 cells and the levels of related cytokines in BHS were higher and the ratio of the Treg cells and the levels of related cytokines in BDS were lower compared to the control group, corresponding results of BSS and BDS group were also have differences, we concluded that the differentiation of immunology on Psoriasis patients can be reflected in TCM blood syndrome.
INTRODUCTION

Psoriasis is a type of scaling erythematous dermatitis with complex etiology and pathogenesis. Recent studies have widely acknowledged that psoriasis is an immune disease under the background of polygenic inheritance disorders with abnormal T cells. The mechanisms by which innate and adaptive immune cytokines regulate inflammation in psoriasis are not completely understood. Many studies have indicated that T helper 17 (Th17) cells played an important role in the development of psoriasis lesion[1]. TNF-α also can stimulate and induce KC to secret the proinflammatory cytokines, which may be another significant pathogenesis of psoriasis.

Regulatory T (Treg) Cells have been known as a major regulator of immune homeostasis through their immunosuppressive function. When activation by stimulation of T cell antigen (TCR), it showed the characteristics of inhibition the proliferation of CD4+ and CD8+ T cells[2]. The transforming growth factor beta (TGF-β) can induce T cells to differentiate into Treg cells, and it is well documented that subsets of Treg produce Interleukin-10 (IL-10) and TGF-β. IL-10 is an important immune mediator, with a multi-effect of anti-inflammatory and immune regulatory function[3]. Several evidence suggest that IL-10 have anti-psoriatic abilities. Retinoic acid-related orphan receptor γ (ROR γ), and forehead box P3 (Foxp3) are the specific transcription factors of Th17 cell and Treg cell, which are the necessary transcription factors for activation[4]. In patients with psoriasis, hyperactivation of Th17 is responsible for abnormalities of the Th17/Treg balance[5], and the impaired function of Treg cells also causes the hyperactivation of Th1 and Th17, each of them can cause psoriatic inflammation[6,7]. Zhang[8] revealed an increasing amount of Th17 and FOXP3+Treg in the blood and lesions in psoriatic patients and a positive correlation with severity of the disease. The correlation of Th17/Treg ratio in skin lesions with PASI was inverse, but in blood it was positive. The imbalance of Treg cells and Th17 cells and each of their main cytokines IL-10/TNF-α, along with the specific transcription factors of Foxp3/ROR γ have played an important role in the pathogenesis of psoriasis with immune injury[9].

Oral systemic medicines have been used successfully for the treatment of psoriasis over 50 years, including methotrexate, ciclosporin, acitretin and fumaric acid esters, however, the highest effective injection of biologics. But most of the oral drugs and biologics for the treatments of psoriasis are limited by their diverse array toxicities. Some data show biologics will associate with serious adverse events and have a risk of infection in first 6 months. Furthermore, biologics agent lack of clinical data to support their long-term effect, safety, even potentially side effect of various organs[10]. Compare with tradition non-biologics systemic and biologics treatment for psoriasis, Traditional Chinese medicine (TCM) treatment for psoriasis characterized by safe, effective, well tolerated, less side effect of long period time.

TCM discriminate psoriasis based on blood syndrome mainly through the area, color, distribution, itch degree and isomorphic response of psoriatic lesion. The classification and treatment of psoriasis vulgaris in TCM are focusing on three TCM syndromes, namely blood-heat syndrome (BHS), blood-stasis syndrome (BSS), and blood-dryness syndrome (BDS)[11]. TCM treated the BHS type with clearing heat and cooling blood method, treated the BSS type with activating blood and resolving stasis method, treated the BDS type with nourishing blood and moistening dryness method. In the early stage most patients have BHS and later it may transform into BSS or BDS. BHS is the key links in the transformation of the pathological changes[11]. So far, there has been little information concerning the relationship between TCM syndromes and T cells and circulating cytokines, although they have been putatively regarded to play important roles in modulating inflammatory responses. In this study, we mainly examined the levels of the Treg/Th17 cells, and related cytokines of IL-10, TNF-specific transcription factors of Foxp3 and ROR γ, So explore the relevant distribution of Treg/Th17 cells between the three TCM syndromes.

MATERIALS AND METHODS

The study was approved by the Beijing University of Chinese Medicine Ethics Committee (Approval No. BJZYDXY-LL-2014017) and written informed consent was obtained for studies involving human participants.

21. Diagnostic criteria and PASI standard: The diagnostic criteria for psoriasis vulgaris were made based on the Guideline for the Treatment of Psoriasis (2008) issued by the Chinese Medical Association[20]. Skin lesion was in accord with the psoriasis area and severity index score standard (PASI) formulated by the Fredrisson Tand Petersson U.

22. TCM standards: The diagnostic criteria for the TCM syndromes of the disease were based on The Differential Diagnostics of TCM Syndrome Complex[21]. The basic syndrome complex: 1) The blood-heat syndrome (BHS): dropped-shape lesion occur and quickly develops, accompanied with erythema in the damaged skin area. New skin lesion appears continuously, with more silvery-white scales, which could not cover the red spots, and could easily strip in the surface and tight-
ly attach to the skin at the bottom; and Auspitz’s sign positive, somorphic response positive. There was severe itching often, accompanied with general symptoms of dry mouth and tongue, constipation, emotional irritability, yellow urine, red or dark-red tongue with thin-white or slightly yellow coating, wry-slippery or rapid pulse. 2. The blood-dryness syndrome (BDS): The duration of the illness was quite long, with rare appearances of new skin lesion in pale-red color. It can be expanded into shape of coin or ring, typically manifests as infiltrated erythema and thin white scaly. Pale tongue with thin-white or little coating, and deep-thready pulse. 3. The blood-stasis syndrome (BSS): The lesion was in dark-red color, and had long course, manifests as infiltrated erythema and thickened white scaly as shell. The dark purple tongue with ecchymotic, and hesitant or uneven pulse.

23. Inclusion criteria: The criteria for patient recruitment included (1) Age between 18 and 65 years old; (2) Willingness to sign an informed consent form; (3) Diagnosis of psoriasis vulgaris with BHS, BSS, and BDS; (4) No co-morbid conditions that might interfere with the results; (5) No hematological abnormality; (6) No active and systemic therapy within a month; (7) No topical application of the corticosteroid or other topical treating medications within 2 weeks.

24. Exclusion criteria: The criteria for excluding patients from the current study were: (1) PASI score standard ≤ 1; (2) Pregnant, lactant women or women who were ready for pregnancy; (3) Exhibition of psoriasis arthritis, pustular psoriasis and erythrodermic psoriasis; (4) With serious systemic disease, cancer, immune deficiency, or mental disorders.

25. Subjects: From the year of 2014, a total of 67 patients from the Beijing Dongzhimen Hospital Dermatology Department were recruited and assigned into one of the three TCM syndrome groups. The distribution of patients is as follows: (1) Gender: BHS group: 22 males (accounted for 55%); 18 females (45%); BSS group: 8 males (57.1%); 5 females (38.5%); BDS group: 8 males (61.5%); 5 females (38.5%); (2) Age: BHS group: 19 to 60 years old (38.25 ± 1.89); BSS group: 23 to 63 years old (43.07 ± 3.59); BDS group: 19 to 64 years old (39.38 ± 2.15); (3) PASI score standard: The severity of disease was graded according to the Psoriasis Area and Severity Index score (BHS group: 15.87 ± 8.02, BSS group: 15.87 ± 8.02, BDS group: 13.17 ± 8.17). Twenty one healthy volunteers (9 males, 12 females) were recruited into the control group.

26. Isolation of human PBMCs: The peripheral blood mononuclear cells (PBMCs) were isolated from the peripheral blood samples of the psoriasis patients and control group by density gradient centrifugation. Cells were collected from the interface, washed with PBS and maintained in RPMI 1640 medium containing 10% FBS and 1% penicillin streptomycin solution.

27. Flow cytometry analysis: The serum levels of the Treg, Th17 cells were measured by a multiplexed flow cytometric assay with Beckman Coulter EPICS XL/FC500/Altra (Bec- kman Coulter, Miami, FL, USA). The cells were stained with CD3-FITC, CD8-ECF and CD4-PC5(BD Pharmingen, USA). CD4/CD25-FITC/PE (Bioscience, San Diego, CA, USA). Prior to IL-17A staining, the cells were incubated for 6h, then suspended in Fixation/permeabilization (Beckman Coulter, Miami, FL, USA), and stained with anti-human IL-17A PE (Bioscience, San Diego, CA, USA), anti-human IFN-γ-FITC (mouse IgGk, 4S.B3, Beckman Coulter, Miami, FL, USA), Foxp3-PE-Cy3 (Bioscience, San Diego, CA, USA). The concentration of cytokines in the peripheral blood was assessed using the special kit (IntraPrepTM Permeabilization Reagent, Cat.No.2388/2389) according to the manufacturer’s instructions.

28. Measurement of cytokines: The peripheral blood samples (3-5mL) taken from the patients and controls were transferred to heparin anticoagulant tubes, followed by centrifugation at 3,000/ min for 10 min. Extracted 400 ul serum was collected and stored at −80℃ for later use. The serum levels of TNF-α, IFN-γ (Human TNF-α, monosay, Catalog HS400C, R&D System, MN, USA), IL-10 (Multi Sciences Biotech, HangZh, China) were measured with the ELISA method according to the manufacturers instructions.

29. RNA isolation and quantitative real-time PCR: We used Primer-BLAST of NCBI to design the primers of target genes. Total RNA was isolated from the cells using an ultra-pure RNA kit (Cwbio. Co. Ltd (Cat# CW0581, Kang Century Biotech Companies, China) and cDNA was synthesized with a first strand cDNA synthesis kit (HiFi-MMLV cDNA) (Cwbio. Co. Ltd, Cat # CW0744). Quantitative real-time PCR was performed using SYBR Green PCR Mixture Cwbio. Co. Ltd (Cat# CW0957, Kang Century Biotech Companies, China). The PCR conditions were incubated at 65℃ for 5 min followed by at 37℃ for 40 min. The results were normalized by an 7500™ RT-PCR apparatus, using the 2−△△Ct method.

210. Statistical analysis: The statistical analysis was performed using the SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). The data were shown as means ± standard deviation, and analyzed with the one-way analysis of variance (ANOVA). Numerical data were compared using the Chi-square test. A P value of less than 0.05 was considered statistically significant. More independent samples through the nonparametric test using the Kruskal-Wallis Test methods.

RESULTS

3.1. Clinical Characteristics: There was no significant difference among the three
TCM syndrome groups and the control group concerning the age, progress, sex ratio or PASI (p>0.05; Table 1).

3.2. Comparison of the ratios of the Treg/Th17 cells between the Three TCM syndrome groups and the healthy control group

The peripheral blood ratios of the Treg cells decreased as compared with the healthy control group. In the BHS and BDS groups the ratios of the Treg cells were found evidently decreased compared with the healthy controls (p=.00;p=.14). There was no statistical difference between the BSS group and the healthy control group (p=.22). There was no difference between the three TCM syndrome groups (p=.17; Table 2; Fig A-1).

In contrary, the peripheral blood ratio of the Th17 cells was increased in the psoriatic patients compared with the healthy control group. In the BHS and BDS groups the ratios of the Th17 cells were significantly elevated compared with the healthy control group (p=.00;p=.00), there was no difference between the BDS group and the healthy group (p=.49). Among the three TCM syndrome groups, the ratio of the Th17 cells in the BHS and BDS groups was insignificantly higher than that in the BDS group (p=.02;p=.01; Table 2; Fig A-2).

3.3. Comparison of serum levels of IL-10, TNF-α among the three TCM syndrome groups and the healthy control group

Serum levels of IL-10 decreased as compared with the healthy control group. In the BHS and BDS groups the serum levels of IL-10 were found evidently decreased compared with healthy control group (p=.00; p=.00), while there was no statistical difference between the BSS group and the healthy control group (p=.10). There was no difference between the three TCM syndrome groups (p=.20; Table 2; Fig B-1).

In contrary, the Serum Level of TNF-α was increased in the psoriatic patients compared with the healthy control group. In the BSS and BDS groups the Serum Level of TNF-α was much higher compared with the healthy control group (p=.00;p=.00), however, there was no significant difference between the BDS group and healthy control group (p=.07). Still there was no difference between the the three TCM syndrome groups (p=.2; Table 2; Fig B-2).

3.4. Comparison of the specific transcription factors of Foxp3mRNA, ROR tmRNA between the three TCM syndrome groups and the healthy control group

The specific transcription factors of Foxp3mRNA decreased compared with the healthy control group. In the BHS group the factor of Foxp3mRNA was significantly lower than the healthy control group (p=.00), as well as in the BDS group (p=.09). No statistical difference was found between the BSS group and the control group (p=.66). Among the three TCM syndrome groups, the levels of Foxp3mRNA was much higher than in the BSS group compared with the BHS and BDS groups (p=.00;p=.01; Table 3; Fig C-1).

The specific transcription factors of ROR tmRNA were increased in the psoriatic patients compared with the healthy control group. The levels of ROR tmRNA were substantially increased in the BHS group.

Table 1. Profile of Four Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Case</th>
<th>Age (Year x±s)</th>
<th>Gender[Case(%)]</th>
<th>Progress (Year x±s)</th>
<th>PASI</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHS</td>
<td>40</td>
<td>38.25±1.89</td>
<td>22(55) 18(45)</td>
<td>13.20±1.37</td>
<td>15.87±8.02</td>
</tr>
<tr>
<td>BSS</td>
<td>14</td>
<td>43.07±3.59</td>
<td>8(57) 6(42.9)</td>
<td>13.36±2.94</td>
<td>13.33±6.61</td>
</tr>
<tr>
<td>BDS</td>
<td>13</td>
<td>39.38±2.15</td>
<td>8(61.5) 5(38.5)</td>
<td>11.23±1.57</td>
<td>13.17±8.17</td>
</tr>
<tr>
<td>Healthy</td>
<td>21</td>
<td>38.29±3.12</td>
<td>9(42.9) 12(57.1)</td>
<td>/</td>
<td>/</td>
</tr>
</tbody>
</table>

Table 2. Comparison of the ratios of Treg/Th17 cells and Serum Levels of IL-10, TNF-α among the Three TCM Syndromes Groups and the Healthy Control Group (pg/mL x±s)

<table>
<thead>
<tr>
<th>mRNA</th>
<th>BHS (11case)</th>
<th>BSS (8case)</th>
<th>BDS (8case)</th>
<th>Healthy control (9case)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foxp3</td>
<td>0.71±0.21**</td>
<td>1.16±0.55**</td>
<td>0.78±0.23**</td>
<td>1.29±0.30</td>
<td>6.33</td>
<td>.00</td>
</tr>
<tr>
<td>RORrt</td>
<td>2.67±0.85**</td>
<td>2.14±0.68</td>
<td>1.66±0.90**</td>
<td>1.23±0.41</td>
<td>6.97</td>
<td>.00</td>
</tr>
</tbody>
</table>

(Note: Compared with Health control group, represent P<0.05; representing P<0.01; Compared with the BHS group, represent P<0.05; Compared with the BDS group, represent P<0.05.)
The peripheral blood ratios of the Th17 cells and Treg assessed by flow cytometry among the Three TCM Syndromes Groups and the Healthy Control Group. The ratios of the Treg cells were found evidently decreased in the BDS and BDS groups compared with the healthy controls (P<0.01;P<0.05;Fig A-1). The ratios of the Th17 in the BDS and BDS groups were significantly elevated compared with the healthy control group (p<0.01;P<0.01; FigA-2), and among the three TCM syndrome groups, the ratio of the Th17 cells in the BDS and BDS groups was insignificantly higher than that in the BDS group (P<0.05;FigA-2).

The serum Levels of IL-10/TNF-α assessed by ELISA among the Three TCM Syndromes Groups and the Healthy Control Group. (B-1 and B-2). The serum levels of IL-10 in the BDS and BDS groups were found evidently decreased compared with healthy control group (P<0.01; P<0.01; FigB-1); The Serum Level of TNF-α in the BDS and BDS groups was much higher compared with the healthy control group (P<0.01; P<0.01; FigB-2) (p=.00). In the BDS group it tends to have higher levels (p=.02). No statistical difference was found between the BDS group and the healthy control group (p=.24). Among the three TCM syndrome groups, the level of ROR-γ tmRNA was elevated in the BDS group compared with the BDS group (p=.01) concurrent with no difference in the BDS group (p=.43; Table3;Fig C-2).

The specific transcription factors of Foxp3mRNA/RORαtmRNA among the Three TCM Syndromes Groups and the Healthy Control Group which assessed by RT-PCR. (C-1 and C-2). The factor of Foxp3mRNA in the BDS group was significantly lower than the healthy control group (P<0.01; FigC-1), as well as the BDS group (P<0.05; FigC-1). The levels of ROR-γ tmRNA were substantially increased in the BDS group (P<0.01; FigC-2), and in the BDS group it tends to have higher levels (P<0.05; FigC-2).

**DISCUSSION**

In this study, we examined the circulating levels of the Treg/Th17 cells and the related inflammatory cytokines (IL-10, TNF-α) along with specific transcription factors (Foxp3, ROR-γ) and found that they were different between the three TCM syndrome groups and the healthy control group, and this provides a clinical guidance for the TCM treatment principle and formula design.

According to the signs and symptoms, TCM has been considered an important approach to deal with psoriasis vulgaris. For example, TCM proposed that the basic pathogenesis of psoriasis was "interior heat accumulation and stagnation of blood". JIN Qi-feng considered that the core pathogenesis of this disease was "heat in blood and excessive toxin". In recent years, researches of TCM syndromes and biological molecular network has established an organic connection between TCM syndrome phenotypes and microscopic biological indicators. The pathogenesis of psoriasis was closely related to the dysfunction of immune cells, which was caused by the imbalance of the immune network. T cell activation has been proven to be the central pathogenesis of immune cells-inflamatory network in psoriasis. The detection of activated CD4+ and CD8+ T cells in the blood can provide useful clinical evidence for diagnosing and evaluating the effect of TCM treatment in psoriasis.

**Table 3. Comparison of the specific transcription factors of Foxp3mRNA, RORαtmRNA among the Three TCM Syndromes Groups and the Healthy Control Group (pg/mL, X±s)**

<table>
<thead>
<tr>
<th></th>
<th>BDS (13 case)</th>
<th>BSS (21 case)</th>
<th>Healthy control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treg</td>
<td>2.47±1.45**</td>
<td>3.56±1.71</td>
<td></td>
</tr>
<tr>
<td>Th17</td>
<td>3.14±1.55**</td>
<td>3.56±2.62**</td>
<td>2.73±1.73</td>
</tr>
<tr>
<td>IL-10</td>
<td>2.41±1.23**</td>
<td>3.24±1.89</td>
<td>1.93±1.29**</td>
</tr>
<tr>
<td>TNF-α</td>
<td>1.65±0.74**</td>
<td>1.79±1.08**</td>
<td>2.60±1.19**</td>
</tr>
<tr>
<td></td>
<td>1.38±0.33</td>
<td>0.93±0.35</td>
<td>5.81</td>
</tr>
</tbody>
</table>

Note: Compared with Health control group, *represent P<0.05; **represent P<0.01; Compared with the BDS group, ▲ ▲represent P<0.01; Compared with the BSS group, represent P<0.05.
CD8⁺ T lymphocytes in the epidermis and dermis of psoriasis is the most favorable evidence.

The Th17 cells are a new type of CD4⁺ T lymphocyte subsets, they can secrete IL-17, IL-6, IL-22, TNF-α, and other cytokines, and mobilize, raise and activate the neutrophils and participate in the occurrence and progression of inflammatory reactions. Increased serum levels of IL-17, IL-23 in psoriasis means the Th17 cells and the associated cytokines were involved in the pathogenesis of psoriasis. In recent years, the Treg cells, independent from the T lymphocyte subsets were found to be different from the Th1 and Th2 cells. TGF-β could motivate native T cells to differentiate into the Treg cells, which mainly secreted interleukin-10 (IL-10) and TGF-β [1]. The Treg cells were differentiat-ed from the initial CD4⁺ T cells, playing the role of mediating immune tolerance while Th17 participating in a series of immune and inflammatory response. The function and the progress of differentiation were in mutual antagonism. Under normal circumstances, they usually maintained homeostasis. Keeping balance of the Th17/Treg cells was essential to maintain immune balance. Clinical and cell experiments have confirmed that imbalance of the Th17/Treg cells might be one pathogenesis of autoimmune diseases [20].

BHS is the most common type of the three TCM syndromes in patients with advanced skin lesions. The study has shown that, in the BHS group, the secretion of the Th17 cells (related cell factors (TNF-α) and specific transcription factors (ROR-γt) were increased, at the same time, the ratio of the Treg cells, interleukin (IL-10) and specific transcription factors (Foxp3) were lower than the normal population (p < 0.05), which means that in this case, the Th17 cells play a dominant role in differentiation, meanwhile Treg cells down-regulation, which indicates that the function of the Th17 cells might suppress Treg cells, leading to the rapid progression of the disease and emergence of new rashes. The BSS always transformed from the BHS and kept latent, and the lesion changed slowly. This experiment showed that in the BSS group, the ratio of the Th17 cells and related factors (TNF-α) and specific transcription factors (ROR-γt) were higher than that in the healthy control group (p < 0.05), whereas the ratio of Treg cells, related factor IL-10 and transcription factors Foxp3 did not show significant difference from the control group (p > 0.05). In this situation, the BSS group was different from the BHS group, the Treg cells and related cytokines stayed normal. We assumed that the Treg cells played a role in regulating the Th17 cells and influenced the related cytokine’s immune function. TCM believes that the pathogenesis of this syndrome is insufficiency of blood with nutritional effect. Specifically, the blockade of qi and its stagnation in the skin, the coexistence of blood stasis and pathogenic toxin and all these appeared to be a struggle between the vital energy and the pathogenic factors. Previous studies showed that [12,23] the Th17 expression level in both BHS and BSS groups was higher than the normal, with most obvious increment in the BHS group. However the Treg cells were not significantly increased at the same time, meaning that the levels of the Th17 cells and related cytokines might become the reference index for the immunological diagnosis and treatment of psoriasis, and the detection of changes in Th17/Treg levels could be used as the objective criteria when assessing the condition of patients. In this study, the results of the Th17 and Treg cells came in line with the previous findings. The BDS could be transformed from BHS and BSS and it was always in a resolution or latent phase clinically. The results of this study confirmed that in the BDS group the levels of pro-inflammatory Th17 cells and cytokine TNF-α, the transcription factor ROR-γt were relatively normal, at the same time, the levels of the Treg cells and cytokine IL-10, the transcription factor Rorγt remained in a declining state. We assumed that dysfunction of the Treg cells might be the main immunological properties of the blood-dryness syndrome.

**Conclusion**

The three TCM syndromes have presented entirely different types of lesion and clinical manifestations. The immune cells of Treg cells and Th17 cells and their relevant specific factors are also in the complex state, as each syndrome has its own key immune indicators. In this experiment we found that the differentiation of Th17 cells accounted for major advantages in BHS, while the Treg cells were evidently decreased in BHS; The differentiation of Th17 cells also accounted for major advantages in BSS, but the Treg cells did not have the significance in BSS. The Treg cells decreased in the immunological characteristics of BDS, while the Th17 cells did not play a practical significance in BDS. This experiment concludes that there is a certain correlation between the blood regulating therapy and the immune balance of Treg/Th17 cells, each syndrome may possess different immune status. The detection of the Treg/Th17 cells and related cytokines or transcription factor to some extent correspond to the biological characteristics of the TCM syndromes. Disorders of immune cells are an important factor in the pathogenesis of psoriasis. Syndrome differentiation and treatment contains of a large amount of objective and microscopic substances, and immune cells and immune network theory may be just part of the whole organism in syndrome differentiation. In research of TCM syndromes as its background, we need to detect the level of various types of cells as much as possible in order to accurately explore the objective law in the classification of TCM syndromes. On the basis of clarifying this point, we can treat psoriasis more pertinently through different therapeutic targets of different Chi-
nese medicines.

REFERENCE


