

Th17/Treg cell level and clinical characteristics of peripheral blood of patients with Sjogren's syndrome complicated with primary biliary cirrhosis

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Abstract

This study aims at analyzing the Th17/Treg cell level and clinical characteristics of the peripheral blood of patients with Sjogren's syndrome (SS) complicated with primary biliary cirrhosis (PBC) so as to deepen the understanding of this disease and seek for its possible onset mechanism.

A retrospective analysis was conducted on the clinical data of 24 patients [8 (33%) males and 16 (67%) females] with SS complicated with primary biliary cirrhosis, 50 patients with primary SS and 93 healthy volunteers. These patients were divided into 3 groups: experimental group (SS+PBC), control group (SS) and healthy group. Then, peripheral blood was collected and flow cytometry was conducted to detect level of Th17 cells and Treg cells. A fully automatic biochemical detector was used to detect the corresponding liver function index. The correlation analysis was made based on the clinical manifestations and biochemical characteristics.

Compared with the healthy group and control group, the experimental group had the highest Th17/Treg cell ratio, and Th17 cell frequency was significantly increased ($P < .05$). Furthermore, ALT, AST, ALP, γ -GT, TBIL, and other indexes were positively correlated to the Th17/Treg ratio ($P < .05$).

Th17/Treg cell level and its ratio in peripheral blood of patients with SS complicated with primary biliary cirrhosis were significantly unbalanced, indicating that Th17 cells participate in the onset of this disease to a large extent. Furthermore, the Th17/Treg ratio has a certain correlation with some of the liver function indexes, on which a stratified analysis could be made furtherly according to the seriousness of the conditions.

Abbreviations: PBC = primary biliary cirrhosis, SS = Sjogren's syndrome, Th17 = T help 17, Treg = regulatory T-lymphocyte.

Keywords: autoimmune liver disease, primary biliary cirrhosis, primary Sjogrn's syndrome, Th17 cells, Treg cells

1. Introduction

The Sjogren's syndrome (SS) is a kind of chronic autoimmune disease that mainly involves the exocrine gland. To date, its onset mechanism remains unclear, and relevant studies have found that infiltrating cells in the labial gland of the target organ are mainly T cells, and most of these are CD4+T cells.^[1] Hence, regulatory

T-lymphocyte (Treg) and T help 17 (Th17) cells have become the T cells of concern in recent years. Some scholars have conducted studies on the measurement of IL-17 and IL-23. By using pathological tissue immunohistochemistry for the pSS mouse model and pSS patients, it was found that the expression was upregulated.^[2] Furthermore, studies have also shown that Th17/Treg imbalance may induce or aggravate the occurrence and development of SS.^[3]

The liver is one of the organs that are easily involved in SS, and several studies have proven that there are 3% to 9% of SS patients who suffer from primary biliary cirrhosis (PBC).^[4] PBC is a kind of autoimmune disease that causes non-suppurative damage in the intrahepatic duct with an unknown cause. In the early stage of the disease, the portal area undergoes a number of autoreactive T cell infiltrations. Even though AMA of the PBC patients was negative, there were still a number of antigen-specific CD4+T cells in the mononuclear cells from the peripheral blood, which could generate IFN- γ , causing the occurrence and development of the disease. Relevant studies have shown that the Th17 cell ratio of PBC patients in PBMCs was increased.^[5] The hepatic pathology revealed that there were Th17 cells gathered around the damaged bile duct. Furthermore, other relevant studies have found that Th17/Treg had a relatively high expression in the PBC liver tissue experiment of patients and animals.^[6,7] Other relevant studies also found that^[8,9] under the stimulation of lipopolysaccharide and other relevant ligands,

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biliary epithelial cells cultured *in vitro* can produce high expression levels of IL-1 β , IL-6, IL-23, and other cell factors to promote Th17 cell differentiation. Hence, researchers have considered that the abnormal increase in Th17 cell frequency would participate in the inflammation development of the bile duct. However, there are few studies on PSS and Th17/Treg expression.

As observed from the above results, Th17/Treg cells participated in the occurrence and development of these 2 diseases. For patients with SS complicated with PBC, the status of Th17/Treg cells is still unknown. Some studies have shown^[10] that PSS and PBC are correlated with each other but they belong to different autoimmune epithelitis subgroups. However, there are no clear studies that could prove that the onset mechanism of both of these is correlated with each other. Recent studies found that Th17 and Treg cells in patients suffering from HBV and other multiple liver diseases exhibited more significant changes when compared to the healthy population,^[6] and that Th17/Treg was considered to be an important immune index that could be used in effective evaluation of the degree of severity of the disease.

The present study detected the levels of peripheral Th17 and Treg cells of these patients, and a correlation analysis was conducted on the clinical characteristics to further specify the influence of Th17/Treg cells on this disease, and search for the possible onset mechanism, which could provide a theoretical basis for targeted therapy in the future.

2. Methods

2.1. General data

In the present study, 24 [8 (33%) males and 16 (67%) females] patients with PBC complicated with SS and 50 [11 (22%) males and 39 (78%) females] patients with SS were selected from the Second Hospital of Shanxi Medical University. These patients were admitted and treated from June 2016 to September 2017. Among these patients, 8 patients were males and 16 patients were females, and their age ranged within 35–79 years old, with an average age of 58 ± 10.7 years old. The average course was 10 months. These patients were divided into 3 groups according to the diagnostic criteria of these 2 diseases: experimental group (SS +PBC), control group (SS), and healthy group (no autoimmune symptoms). This study was conducted in accordance with the declaration of Helsinki and approved by Ethics Committee of the Second Hospital of Shanxi Medical University. Written informed consent was obtained from all participants.

2.2. Diagnostic criteria

The diagnostic criteria for PBC were based on the guidelines for the diagnosis and treatment of PBC prepared by the American Association for the Study of Liver Diseases. SS was diagnosed on the basis of the international classification (diagnosis) criteria (2002) for SS. The interference of external sarcoidosis, lymphoma, AIDS, xerosis cornea, or parotitis due to known causes and other symptoms are excluded.

2.3. Sample collection and cell separation

The peripheral blood obtained from qualified patients and the population in the healthy group was extracted through the vein

and collected in 2 tubes, and blood volume in each tube was 3 mL. For the first tube, the density gradient centrifugation method was used to separate the PBMC, and the RPMI1640 culture solution was used to adjust the PBMC density. For the second tube, plasma was collected for ELISA detection.

2.4. Detection index

A flow cytometer (BD FACSCalibur) was used to detect the frequency of Th17 and Treg cells in peripheral blood. An automatic biochemical analyzer (Beckman Inc.; AU5800) was used to detect the serum TB, AST, ALP, γ -GT and TBIL levels, and other relevant clinical data.

2.5. Statistical analysis

SPSS 18.0 statistical software was used for statistical treatment. In the present study, all results were expressed as mean \pm standard deviation. For 3 sets of data, independent sample *t* test was carried out. Based on the inspection standard, $P \leq .05$ was considered statistically significant.

3. Results

3.1. Clinical manifestations

As shown in Table 1, the clinical manifestations of patients revealed the following: There were more males (33%) in SS patients with PBC than those in controls (22%); In the experimental group, 24 patients had jaundice, skin itch, ascites, and other hepatic pathological changes, and the proportion of the main clinical symptoms was significantly increased, when compared to the control group. Furthermore, the proportion of patients suffering from weakness was basically consistent with that in the control group, but the proportion of patients suffering from loss of weight was higher in the control group than that in the experimental group.

3.2. Examination results of liver function and immune indexes

As shown in Table 2, the ALT, AST, ALP, γ -GT, and TBIL of patients in the experimental group were higher than those in the control group and healthy group upon statistical calculation ($P < .05$). The relevant indexes of patients in the control group were also significantly higher than those in the healthy group.

3.3. Examination results of Th17, Treg, and the Th17/Treg ratio

As shown in Figure 1A, the Th17 cell frequency in the experimental group was $14.39 \pm 7.11\%$, which was significantly higher than that in the control group ($11.93 \pm 6.04\%$), while the frequency in the healthy group was $6.54 \pm 4.04\%$. However, Treg cell frequencies were slightly changed, which were as follows: $30.55 \pm 22.53\%$ in the experimental group, $29.43 \pm 19.57\%$ in the control group, and $27.67 \pm 13.96\%$ in the healthy group. As shown in Figure 1B, the Th17/Treg ratio in the experimental group was the highest, which was 0.46 ± 0.27 . This was higher than that in the control group (0.40 ± 0.33) and healthy group (0.25 ± 0.15). Upon calculation, the difference was statistically significant ($P < .05$).

Table 1

The distribution of main clinical manifestations between the experimental group and the control group.

Clinical symptom	Groups			
	Experimental group (n=24)		Control group (n=50)	
	n	Percent, %	n	Percent, %
Jaundice	17	70.8	12	24.0
Skin itch	10	41.7	19	38.0
Anorexia	12	50	2	4.0
Hepatomegaly	14	58.3	6	12.0
Digestive tract hemorrhage	5	20.1	5	10.0
Dry mouth	20	83.3	32	64.0
Joint gall	23	95.8	42	84.0
Oesophageal varices	4	16.7	3	6.0
Fever	9	37.5	2	4.0
Ascites	8	33.3	6	12.0
Abdominal distension	14	66.7	9	18.0
Weight loss	21	87.5	48	96.0
Feeble	23	95.8	45	90.0

4. Discussion

The symptom of SS complicated with PBS is not uncommon.^[11,12] Through the classification comparison of the experimental group and control group in the present study, the results revealed that the number of patients suffering from jaundice, skin itch, hepatomegaly, and other clinical symptoms in the control group was lower than that in the experimental group, and ALT, AST, ALP, and other liver function indexes of these 2 groups also had a significant difference. Furthermore, the data presented in the experimental group was the most significant.

Relevant studies have considered that^[13] the tubule peripheral inflammatory infiltration in patients (SS+PBC) and the class II HLA molecule of the epithelial cell surface have an abnormal expression, and for patients with SS, the frequency of the abnormal expression of HLA cells is relatively lesser. Most of the bile ducts and glands of patients with SS+PBC involve CD4⁺T cell infiltration, making the liver and other exocrine glands have a common antigenicity. Hence, these become the target organs of the autoimmune response of patients with PSS+PBC, thereby increasing relevant function parameters.

Table 2

The comparison of liver function indexes among the 3 groups ($\bar{x} \pm s$).

Groups	n	ALT, U/L	AST, U/L	ALP, U/L	γ -GT, U/L	TBIL, μ mol/L
Experimental group	24	72.31 \pm 9.51	112.47 \pm 74.26	218.56 \pm 94.26	252.43 \pm 96.82	65.29 \pm 58.25
Control group	50	58.24 \pm 13.57	55.69 \pm 34.26	160.38 \pm 44.54	194.69 \pm 127.25	54.87 \pm 36.91
Healthy group	93	24.91 \pm 7.94	24.17 \pm 10.75	78.89 \pm 20.14	42.61 \pm 14.92	26.27 \pm 14.36
P	-	<.05	<.05	<.05	<.05	<.05

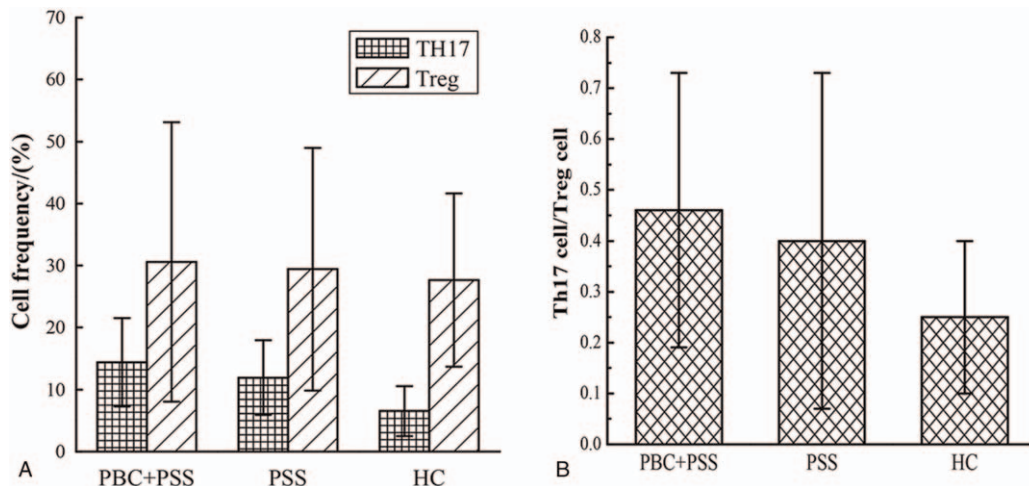


Figure 1. Comparison between Th17 and Treg cell frequencies, and the Th17/Treg ratio.

Table 3
The comparison of albumin and coagulation function indexes among the 3 groups ($\bar{x} \pm s$).

Groups	n	ALB, g/L	PT, s	APTT, s	INR
Experimental group	24	38.24±4.23	16.79±2.76	45.61±12.25	1.37±0.29
Control group	50	34.63±10.28	15.89±6.16	44.29±10.92	1.35±0.63
Healthy group	93	28.51±3.21	10.8±3.23	33.41±5.12	1.05±0.21
P	–	<.05	<.05	<.05	<.05

In addition, under the steady state or non-inflammatory state, and immune disorder, the human body's Th17 and Treg cells maintain its balance.^[14] However, in this experiment, the proportion of peripheral Th17 and Treg cells in the experimental group was higher than that in the control group. Although the frequency of Treg cells in patients with SS was slightly lower, the Treg levels in both groups were basically the same. Above data indicate that relative increase in pro-inflammatory cells is associated with the pathological basis of these 2 kinds of symptoms.

Th17 cells can secrete the IL-17 proinflammatory factor during immune expression, which induces matrix metalloproteinase (MMP), and produces osteoclast,^[15] which further damages liver function. At the same time, Tregs can exert an immunoregulatory effect by releasing inhibitory cytokines IL-10 and TGF- β ^[16] to suppress the inflammatory immune response. Under the single action of TGF- β , the initial T cell is induced to be differentiated into Tregs, while under the common action of TGF- β and IL-6, the initial T cell is induced to be differentiated into TH17 cells.^[17] Notably, TH17 and Treg cells transform with each other under a specific cytokine environment.^[18] Furthermore, although the mechanism of SS with liver disease is unclear, our results showed that the increased proportion of Th17 cells in patients with SS+PBC resulted in the local immune dysfunction, causing Th17/Treg imbalance. This shows that the Th17 cell function of patients with SS+PBC was the dominated, and the secreted IL-17 would further accelerate the inflammatory response, causing an increase in liver function indicators and other indexes, and making these clinical manifestations more significant. The Th17/Treg ratio of patients with SS was higher than that in the healthy group, but this was still lower than that in the experimental group, indicating a low level of this imbalance as well as showing that the initial T cell differentiation in the body was abnormal. This might be due to the fact that the level of specific transcription factor Foxp3 in Treg cells was increased to inhibit the secretion of ROR γ t- key nuclear factor of Th17 cell,^[19] which leading to the decline of the Th17 cell frequency. However, due to the immune dysfunction, its ratio in experimental group remained higher than that in the healthy group.

The changes in serum albumin and coagulation function in peripheral blood were closely related to the degree of liver function and prognosis.^[20] Through comparison on the albumin and other coagulation function indexes of patients between both non-healthy groups and the healthy group (Table 3), and in addition to the notion that the ALB index in the experimental group was relatively high, other data were within the scope of a normal value, showing that the coagulation function of patients with PSS+PBC was affected to a certain extent.

Our findings reveal the important characteristic of immunopathology: the imbalance of Th17/Treg cells in SS patients

complicated with PBC helps further understanding of the pathology of SS. The correlation between Th17/Treg levels and clinical features in SS patients complicated with PBC deepens the understanding of the disease. Elevated proportion of peripheral Th17 cells may be used as indicator for the diagnosis and disease severity of SS complicated with PBC and even as potential target of therapy.

The present study only researched on the correlation between Th17/Treg and SS and PBC diseases, and the results revealed differences in Th17/Treg in patients with SS+PBC and patients with SS. These indicate that there was a certain correlation between Th17/Treg and the experimental samples. At present, there are few similar domestic studies, and it is difficult to provide an accurate reason for the changes in the differences between Th17 and Treg. Some studies have shown^[10] that SS and PBC are correlated to each other but they belong to different subgroups with autoimmune epithelitis. However, there are no clear studies that could prove that the onset mechanism of both of these is correlated with each other. Researchers should adopt experiments so as to further determine that Th17 cells make more sense in patients with SS complicated with PBC, which provides a reference for further research on the onset mechanism and treatment of this disease.

Author contributions

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