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Expression of Helper T Cell Type 17 and CD4+ CD25+ Tregs in AMA-M2 Positive PBC Patients

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Abstract: *Objective*: To investigate the expression and impact of helper T cell type 17 and $CD4^+$ $CD25^+$ regulatory T (Treg) cells in anti-mitochondrial M2 antibody (AMA-M2) positive primary biliary cholangitis (PBC) patients. *Methods:* Thirty PBC patients with positive AMA (M2 type) (antibody titer above 1:320) by indirect immunofluorescence assay under the Affiliated Hospital of Hebei University from November 2021 to August 2022 were selected as the experimental group, while 30 healthy individuals were selected as controls. The subjects were observed and analyzed for AFP-L3 and immunoglobulin expression. *Results:* The levels of Thl7, Treg, Thl7/Treg, interleukin (IL)-17A, IL-2, IL-10, and transforming growth factor (TGF)-β1 cytokines of the experimental group were 2.61 ± 0.48, 1.15 ± 0.54, 2.41 ± 0.47, 310.94 ± 21.14, 276.36 ± 36.12, 317.89 ± 28.97, and 197.48 ± 31.04, respectively, while those of the control group were 1.14 ± 0.58, 0.88 ± 0.29, 1.47 ± 0.25, 9.69 ± 1.26, 57.69 ± 2.45, 154.01 ± 19.87, and 514.36 ± 36.12, respectively, wherein $P < 0.05$; the $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ of the experimental group were 39.48 ± 4.19, 20.12 ± 4.41, and 1.76 ± 0.14, respectively, while those of the control group were 35.78 ± 4.21, 22.01 ± 4.16, and 1.51 ± 0.13, respectively, wherein $P < 0.05$. *Conclusion:* In patients with PBC, there is a significant imbalance in Thl7/Treg cells. Il-17A, IL-2, IL-10, and TGF- β1 cytokines play important roles in the differentiation and functional expression of both Thl7 and Treg cells.

Keywords: Helper T cells 17; Treg cells; Primary biliary cholangitis

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1. Introduction

Primary biliary cholangitis (PBC), once known as primary biliary cirrhosis (PBC), is a chronic progressive autoimmune cholestatic disease, in which the liver is the primary target organ. The main pathological change in PBC is manifested as non-suppurative inflammation of small intrahepatic bile ducts, eventually leading to liver fibrosis and cirrhosis. PBC mainly affects middle-aged and elderly women and is characterized by high titers of anti-mitochondrial antibodies in the serum (AMA), elevated bile enzymes, and characteristic hepatic pathological changes. This disease is mainly caused by genetic and environmental factors; however, its pathogenesis is unknown, its clinical manifestations are insidious, and some patients have already developed cirrhosis by the time the disease is detected. PBC is often associated with high titers of anti-mitochondrial antibodies (AMA), which tend to appear early in the course of the disease. AMA is an antibody against lipoprotein components of the inner mitochondrial membrane, without organ or species specificity. AMA can be any of the five immunoglobulins. It is an autoantibody found primarily in the serum of patients with primary biliary cholangitis. It is commonly used to aid the diagnosis of jaundice and determine the etiology of liver disease. A positive result is seen in primary biliary cirrhosis. More than 50% of such patients have serum antimitochondrial antibody titers (that is, the dilution of the serum) of 1:200 to

1:3200. In addition, it may also be positive in autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis, progressive systemic sclerosis, and desiccation syndrome. Detecting the expression of T helper cell 17 and $CD4^+$ $CD25^+$ Treg cells in the peripheral blood of patients with AMA-M2 positive PBC enables further exploration of the mechanism of action between pro-inflammatory Th17 cells and inhibitory Treg cells and provides a reasonable basis for early accurate diagnosis and further treatment of patients with AMA-M2 positive PBC.

2. Methods

2.1. General information

Thirty patients with PBC who were positive for AMA (M2 type) (antibody titer 1:320 or higher) by indirect immunofluorescence assay from November 2021 to August 2022 under the Affiliated Hospital of Hebei University were selected as the experimental group, while 30 healthy individuals were selected as controls.

2.2. Methodology

(1) The number of Th17 cells and $CD4^+$ $CD25^+$ Treg cells in the experimental and control groups were analyzed by flow cytometry, and the expression of CD25, CD17, and FoxP3 in this cell subpopulation was analyzed by flow cytometry of the selected T lymphocyte subpopulation of $CD3^+$ $CD4^+$.
(2) The expression of transcription factors RORγt, STAT3, and p-RORγt was detected by protein immunoblotting.
(3) The levels of interleukin (IL)-17, IL-2, IL-10, and transforming growth factor (TGF)-β1 were measured by enzyme-linked immunosorbent assay.

2.3. Statistical analysis

Each experiment was repeated three times. All experimental data were analyzed by SPSS 21.0, and the measurement data were expressed as mean ± standard deviation ($\bar{x}$ ± s), and one-way analysis of variance (ANOVA) was used to compare the differences between multiple groups, and Student–Newman–Keuls (SNK)-q test was used for two-way comparison. The difference was considered statistically significant when $P < 0.05$.

3. Results

3.1. Comparison of Thl7 and Treg cell ratios and cytokine levels

The Thl7, Treg, Thl7/Treg, IL-17A, IL-2, IL-10, and TGF-β1 cytokine levels of the experimental group were 2.61 ± 0.48, 1.15 ± 0.54, 2.41 ± 0.47, 310.94 ± 21.14, 276.36 ± 36.12, 317.89 ± 28.97, and 197.48 ± 31.04, respectively, while those of the control group were 1.14 ± 0.58, 0.88 ± 0.29, 1.47 ± 0.25, 9.69 ± 1.26, 57.69 ± 2.45, 154.01 ± 19.87, and 514.36 ± 36.12, respectively, wherein $P < 0.05$ (**Table 1**).

Table 1. Comparison of Thl7 and Treg cell ratios and cytokine levels

Groups	Experimental group (n = 30)	Control group (n = 30)	*P*
Thl7	2.61 ± 0.48	1.14 ± 0.58	< 0.05
Treg	1.15 ± 0.54	0.88 ± 0.29	< 0.05
Thl7/Treg	2.41 ± 0.47	1.47 ± 0.25	< 0.05
IL-17A	310.94 ± 21.14	9.69 ± 1.26	< 0.05
IL-2	276.36 ± 36.12	57.69 ± 2.45	< 0.05
IL-10	317.89 ± 28.97	154.01 ± 19.87	< 0.05
TGF-β1	197.48 ± 31.04	514.36 ± 36.12	< 0.05

3.2. Comparison of peripheral blood helper T lymphocyte subpopulations between the two groups

The $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ of the experimental group were 39.48 ± 4.19, 20.12 ± 4.41, 1.76 ± 0.14, respectively, while those of the control group were 35.78 ± 4.21, 22.01 ± 4.16, and 1.51 ± 0.13, respectively, wherein $P < 0.05$ (**Table 2**).

Table 2. Comparison of peripheral blood helper T lymphocyte subpopulations between the two groups

Groups	$CD4^+$	$CD8^+$	$CD4^+/CD8^+$
Experimental group (n = 30)	39.48 ± 4.19	20.12 ± 4.41	1.76 ± 0.14
Control group (n = 30)	35.78 ± 4.21	22.01 ± 4.16	1.51 ± 0.13
P	< 0.05	> 0.05	< 0.05

4. Discussion

The liver is a special organ that plays a key role in inducing immunological tolerance. Patients with hepatitis B virus (HBV) infection have certain defects in both, innate and acquired immunities. HBV infection itself does not have a direct damaging effect on hepatocytes but indirectly injures hepatocytes by inducing an autoimmune response in the host. Such immune-related factors have a more significant impact on the regression and prognosis of hepatitis B patients. In addition, alpha fetoprotein (AFP)-L3 is an AFP heterodimer produced only in cancer cells. In 2005, AFP-L3 has been recognized by the United States Food and Drug Administration (US FDA) as an early warning marker for diagnosing liver cancer. In this context, the aim of this paper was to analyze the expression of helper T lymphocytes, AFP-L3, and immunoglobulins in the serum of patients with hepatitis B cirrhosis and its value for prognostic assessment, so as to provide a basis for the clinical assessment of the prognosis of patients with hepatitis B cirrhosis.

Disruption of the balance between pro-inflammatory Th17 cells and suppressive Treg cells is a key factor in the pathogenesis of autoimmune diseases. Th17 cells specifically produce IL-17 effectors that mediate inflammatory responses and autoimmune reactions. $CD4^+$ $CD25^+$ Treg cells are considered an important subclass of regulatory T cells that have strong immunosuppressive effects. They also play important roles in maintaining peripheral tolerance and preventing the development of autoimmune diseases [1–6].

PBC is a global disease with approximately 10 times the number of women than men, and this disease is not uncommon in China. In 2010, an epidemiological study of PBC in China reported a prevalence of 49.2 per 100,000, with a prevalence of 155.8 per 100,000 in women over 40 years of age. With increasing awareness of the disease in recent years, many patients with early-stage disease are being diagnosed. These patients have liver pathology that is still in a small bile duct inflammatory state and respond relatively well to treatment.

There are many factors involved in the pathogenesis of cirrhosis, including cytokines. An increasing number of studies have now shown that the pathogenesis of cirrhosis involves various signaling pathways, among which the TGF-β/Drosophila mothers against decapentaplegic protein (SMAD) signaling pathway plays an important role. A large amount of activated TGF-β can activate the SMAD signaling pathway, which not only induces the activation of quiescent hematopoietic stem cells (HSCs) to differentiate into fibroblasts (desmocytes), but also promotes the gene transcription of extracellular matrix (ECM) protein synthesis while inhibiting collagenase catabolism, leading to the onset and progression of liver fibrosis, and eventually cirrhosis [7–9]. IL-10 is an inflammatory factor secreted by dendritic cells and other antigen-presenting cells [10]. It promotes Thl7 cell differentiation along with TGF-P and IL-6 and not only directly participates in the induction of $CD4^+$ T lymphocytes into Thl7 cells, but also activates memory cells to produce cytokine IL-17, which has an important role in the proliferation of Thl7 cells [11,12]. Studies have

shown that the differentiation of Thl7 cells reduced with IL-17 levels in IL-10 knockout mice [13]. Previously, IL-2 was thought to be produced by T follicular helper cells, with some secreted by $CD4^+$ T lymphocytes, $CD8^+$ T lymphocytes, and natural killer T cells; in autoimmune and infectious diseases, Thl7 cells can also secrete IL-2, thus contributing to tissue damage and disease activity [14]. For instance, in the course of chronic hepatitis B (CHB), the IL-2 secreted by Thl7 cells can also promote their own differentiation, creating a positive feedback effect; in the absence of IL-2, TGF-p and IL-6 are less effective in inducing differentiation of Thl7 cells [15]. TGF-β is a multifunctional cytokine in the transforming factor superfamily. It can be secreted by Treg cells, neutrophils, macrophages, *etc*. It has three isoforms, of which TGF-βl has the highest level in liver tissue. TGF-βl has a dual role in the regulation of the immune system by HBV infection. On the one hand, it stimulates the differentiation of Thl7 cells, thus regulating the inflammatory state of the liver; on the other hand, it plays an important role in the differentiation of Treg cells, which have an anti-inflammatory function. When Treg cells are more inclined to a certain cell differentiation, there may be varying outcomes, implying that Treg cells play an important role in the fate of HBV infection. TGF-β1 can also upregulate hepatocyte nuclear factor-4α (HNF-4α) in hepatocytes to exert its anti-HBV effect. Other than regulating HBV replication, HNF-4α can also improve the degree of liver fibrosis.

While the levels of IL-17A, IL-2, and IL-10 continued to rise, which may be specific cytokines expressed in the imbalance between Thl7 cells and Treg cells, the level of TGF-β1 decreased in the three groups. This may be related to the use of antiviral drugs in both, chronic hepatitis B group and hepatitis B cirrhosis group, as well as the continuous consumption of antiviral drugs in the degradation process of HBV covalently closed circular (ccc)DNA caused by cytosine nucleoside deaminase deamination. Activation-induced cytidine deaminase (AID) achieves deamination by triggering the targeting effect of uracil-DNA glycosylase. Relevant studies have shown that there is no significant difference in peripheral TGF-β1 levels between normal healthy individuals and chronic hepatitis B patients without antiviral treatment. This may be explained by the fact that TGF-β1 is secreted not only by Treg cells, but also by other cells.

Peripheral blood $CD4^+$ and $CD4^+$/CD8 percentages were higher in the cirrhosis group than in the control group and were lower in the carcinoma group than in the control and cirrhosis groups. The percentage of peripheral blood $CD8^+$ was higher in the cirrhosis group than in the control group, while the percentage of peripheral blood $CD4^+$ was higher in the carcinoma group than in the control group. Based on the correlation analysis results, peripheral blood $CD8^+$ percentage was found to be significantly negatively correlated with the prognosis of hepatitis B cirrhosis, *i.e*., the higher the $CD8^+$ percentage, the poorer the prognosis. These findings are consistent with previous studies demonstrating persistent HBV infection in patients with post-hepatitis B cirrhosis and liver cancer, as seen by their consistently low peripheral $CD4^+$ and $CD4^+/CD8^+$ levels. The difference in the expression intensity of TH-related indicators *in vivo* may be the primary reason for the varying prognosis of patients with hepatitis B cirrhosis.

In conclusion, there is a significant imbalance in Thl7/Treg cells in patients with primary biliary cholangitis. Furthermore, Il-17A, IL-2, IL-10, and TGF- β1 cytokines play important roles in the differentiation and functional expression of both Thl7 and Treg cells.

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Disclosure statement

The authors declare no conflict of interest.

References

[1] Wu Y, Chen S, Yang C, et al., 2022, Effect of Interleukin-23 Receptor Overexpression on the Balance of Helper T Cell 17/Regulatory T Cells in Mice with Experimental Autoimmune Uveitis. Chinese Journal of Ophthalmology, 38(05): 389–395.

[2] Zhang Z, Gan M, 2022, Significance of Serum FGF19, Klotho and FGFR4 Protein Expression in Patients with Primary Biliary Cirrhosis. Chinese Primary Medicine, 29(05): 660–663.

[3] Sun Y, Wang H, Zhang R, et al., 2022, Effect of Tonic Lung Huayu Tang Combined with Albumin Paclitaxel + Cisplatin Chemotherapy Regimen on the Levels of Squamous Cell Carcinoma Antigen and Helper T Lymphocyte 17/Regulatory T Lymphocytes in Patients with Advanced Squamous Lung Cancer. Hebei Chinese Medicine, 44(03): 420–424.

[4] Zeng Y, Li G, Li H, et al., 2022, Efficacy of Ursodeoxycholic Acid in the Treatment of Patients with Primary Biliary Cholangitis and the Effect on Serum Nrf2 and HO-1 Levels. Journal of Practical Liver Diseases, 25(02): 211–214.

[5] Su Q, Wang Y, Chen H, et al., 2021, Clinical Characteristics of 122 Cases of Autoimmune Hepatitis-Primary Biliary Cholangitis Overlap Syndrome. Journal of Tropical Medicine, 21(07): 916–920 + 949.

[6] Liu Y, Liu H, 2021, Advances in the Study of Molecular Regulatory Mechanisms of Pathogenicity of Helper T Cell 17 Cells. Journal of Modern Traditional Chinese and Western Medicine, 30(21): 2389–394.

[7] Wang Y, Su R, Guo Q, et al., 2021, Association Analysis of Peripheral Blood Helper T Cell 17/Regulatory T Cell Imbalance with Cytokines in Patients with Immunoglobulin G4-Associated Diseases. Chinese Journal of Rheumatology, 25(04): 217–224.

[8] Zhang T, Xu W, Zhou S, 2021, Efficacy of Prednisone Combined with Hesperidin Sodium in the Treatment of Facial Neuritis and the Effect on Regulatory T Cells/Helper T Cells 17 Cytokines and Free Radical Production. Chinese Pharmacoeconomics, 16(03): 95–98.

[9] Li W, Zhou Y, Zhang X, 2020, Peripheral Blood Hypoxia-Inducible Factor-1α, Regulatory T Cells and Helper T Cells 17 and Inflammatory Cytokine Levels in Patients with Nonalcoholic Fatty Liver Disease and Their Correlation. Chinese Journal of Liver Diseases, 12(04): 83–88.

[10] Yang J, Li N, 2020, Advances in the Study of Adjuvant T Lymphocyte 17 in Chronic Inflammatory Liver Disease. Journal of Clinical Hepatobiliary Diseases, 36(12): 2808–2810.

[11] Lin Z, Bian Y, Ma S, et al., 2020, Genome-Wide Microarray-Based Differentiation Study of Helper T Cells 17 in Rats with Experimental Arthritis. Chinese Journal of Clinical Pharmacology, 36(20): 3317–3320.

[12] Li X, 2020, Study on the Expression Level of High Mobility Group Protein B1 on Peripheral Blood of Patients with Primary Dry Syndrome and the Correlation with Helper T Cell 17/Regulatory T Cells, Shanxi Medical University.

[13] Xue M, Mao X, Chen H, 2020, Characteristics of Changes in Auxiliary T Lymphocyte 17/Regulatory T Lymphocyte Ratio Imbalance in a Rat Model of Immune Liver Injury. Journal of Clinical Hepatobiliary Diseases, 36(01): 149–152.

[14] Schmidt T, Chen L, Li W, 2019, Frequency of Helper T Lymphocyte 17 Is Associated with Low Bone Mass in Primary Sclerosing Cholangitis. Journal of Clinical Hepatobiliary Diseases, 35(03): 569.

[15] Li X, Shi Y, Yang X, 2018, Effect and Significance of Gemcitabine on Helper T-Cell 17/CD4+CD25+ Regulatory T Cells and Tumor-Associated Macrophages in Patients with Cervical Cancer. Anhui Medicine, 22(11): 2245–2250.