Alterations in Gamma-Delta T Cells in Patients With Primary Biliary Cholangitis

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Abstract

Background & Aims

Gamma-delta ($\gamma\delta$) T cells are involved in the development of diverse liver and autoimmune diseases, whereas the role of $\gamma\delta$ T cells in primary biliary cholangitis (PBC) remains unclear.

Methods

We analyzed the number, phenotypes, and functional molecules of $\gamma\delta$ T cells in PBC patients ($n = 74$) and sex- and age-matched healthy controls (HCs) ($n = 74$) by flow cytometric analysis.

Results

We identified two distinct functional subsets of circulating $\gamma\delta$ T cells according to the CD3/TCR $\gamma\delta$ complex: the TCR $\gamma\delta^{\text{high}}$ and TCR $\gamma\delta^{\text{low}}$ subsets. Approximately three-quarters of cells in the TCR $\gamma\delta^{\text{high}}$ subset were V δ 1 T cells, while V δ 2 T cells were enriched in the TCR $\gamma\delta^{\text{low}}$ subset in HCs. The frequency and absolute number of circulating TCR $\gamma\delta^{\text{low}}$ cells was significantly decreased in PBC patients compared with HCs ($p < 0.001$). Furthermore, the frequency of TCR $\gamma\delta^{\text{low}}$ cells was negatively correlated with disease severity and positively correlated with the ursodeoxycholic acid response. TCR $\gamma\delta^{\text{low}}$ cells exhibited a similar apoptotic and proliferative phenotype but enhanced liver-homing chemokine receptor (CXCR6) expression in PBC patients compared with HCs. In addition, both TCR $\gamma\delta^{\text{high}}$ and TCR $\gamma\delta^{\text{low}}$ subsets were more activated in PBC compared with HCs, characterized by elevated expression levels of CD69 and HLA-DR. Finally, we found an increased granzyme B (GZMB) production and similar IFN- γ and TNF- α production of TCR $\gamma\delta^{\text{low}}$ cells in PBC patients compared with HCs.

Conclusion

The TCR $\gamma\delta^{\text{low}}$ subset might be a potential marker for disease progression and treatment response in PBC, which may play a crucial role in liver injury through increased CXCR6 expression and GZMB production.

Introduction

Primary biliary cholangitis (PBC) is an autoimmune liver disease with an increasing tendency of prevalence worldwide[1]. Although the pathogenesis of PBC has not been fully clarified, innate and adaptive immune responses play a key role in liver injury[2].

Gamma-delta ($\gamma\delta$) T cells are a unique population of unconventional T cells with γ and δ glycoprotein chains. In humans, $\gamma\delta$ T cells represent 2–10% of T cells in the peripheral blood and 5–15% of T cells in

the liver, which regulates inflammation, pathogen clearance, and tumor immunity through the production of various cytokines[3–7]. Previous studies have demonstrated that $\gamma\delta$ T cells are closely involved in the development of viral hepatitis and autoimmune liver diseases[5, 8, 9]. However, the frequency of $\gamma\delta$ T cells in PBC remains controversial in previous studies with small sample sizes[9–12]. The function of $\gamma\delta$ T cells in PBC also needs to be further explored.

Additionally, most works on human $\gamma\delta$ T cells have focused on V δ 1 and V δ 2 T cells, which are discriminated by different types of γ and δ chains[4]. Yokobori N et al. reported a novel way to identify two distinct subsets of $\gamma\delta$ T cells, called the TCR $\gamma\delta^{\text{high}}$ and TCR $\gamma\delta^{\text{low}}$ subsets, in patients with tuberculous pleurisy according to CD3/TCR $\gamma\delta$ complex expression[13]. In line with this, a recent study found different expression levels of transcription factors, including PLZF and ROR γ t, between human circulating TCR $\gamma\delta^{\text{high}}$ and TCR $\gamma\delta^{\text{low}}$ cells[14], indicating that the two distinct subsets might have different phenotypes and functions. However, the role of the TCR $\gamma\delta^{\text{high}}$ and TCR $\gamma\delta^{\text{low}}$ subsets in PBC remains unclear.

Therefore, our study aimed to explore the number, phenotype, and functional molecules of $\gamma\delta$ T cells in PBC, with a special focus on the TCR $\gamma\delta^{\text{high}}$ and TCR $\gamma\delta^{\text{low}}$ subsets.

Patients And Methods

Patients

PBC patients were diagnosed and enrolled at Beijing Friendship Hospital, Capital Medical University. This study was approved by the Institutional Committee for Human Research of Beijing Friendship Hospital, and written informed consent was obtained from all participants.

Patients will be included if they (1) were diagnosed with PBC based on international diagnostic criteria[2, 15] and (2) were treatment-naïve or received monotherapy with UDCA at a dose of 13–15 mg/kg/day. Patients were excluded if they (1) had coexistent other liver diseases, such as autoimmune hepatitis, chronic hepatitis B or C infection, drug-induced liver injury, alcoholic liver disease or metabolic fatty liver disease; (2) had coexistent malignant tumors, diabetes, or other autoimmune diseases, such as Sjogren's syndrome or autoimmune thyroiditis; or (3) were treated with corticosteroid or immunosuppressive drugs in the last 4 weeks.

Healthy controls (HCs) were included if they (1) were age- and sex-matched with PBC patients and (2) had normal routine blood, liver and kidney function, glucose, and lipid test results, routine urine test results, and abdominal ultrasound results in the last six months. HCs were excluded if they (1) had coexistent liver diseases, autoimmune diseases, diabetes, tumors or other diseases that can shorten life expectancy or (2) suffered from alcohol abuse.

Blood sample collection and PBMC preparation

Blood samples were obtained from 74 PBC patients and 74 healthy volunteers. Peripheral blood mononuclear cells (PBMCs) were freshly isolated by Ficoll-Hypaque density centrifugation.

Analysis of the number and phenotypes of $\gamma\delta$ T cells

Flow cytometry was performed using the following antibodies: anti-CD3-Brilliant Violet 711, anti-TCR $\gamma\delta$ -PE, anti-TCRV δ 2-FITC, anti-CXCR6- PE/Cy7, anti-CXCR3- PE/Cy7, anti-CD69- PE/Cy7, anti-HLA-DR-APC/Cy7, anti-CD25- PE/Cy7, anti-Annexin V (AV)-FITC, and anti-Ki67-APC, which were purchased from Biolegend. Anti-TCRV δ 1-PE/Cy7 was obtained from Thermo Scientific (Waltham, MA, USA).

Flow cytometry was performed using a FACS Aria II flow cytometer (BD Biosciences, CA, USA), and the data were analyzed with FlowJo software (Treestar, Ashland, OR, USA).

Detection of the functional molecules of $\gamma\delta$ T cells

For the detection of cytokines, PBMCs were cultured in complete medium (RPMI 1640, 10-040-CV, Corning, supplemented with 100 U/mL penicillin, 100 μ g/mL streptomycin, 10% FBS, and 2 mM glutamine) with phorbol myristate acetate (PMA)-ionomycin cocktail (Biolegend) at 50 ng/ml for 4 hours at 37 °C. Antibodies (anti-GZMB-FITC, anti-TNF- α -APC, and anti-IFN- γ -PE/Cy7) were purchased from Biolegend.

Statistical analysis

Categorical variables are expressed as counts and percentages. Continuous variables are all described as medians with interquartile ranges since they did not fit the normal distribution. The Mann–Whitney U-test was performed to compare the differences in continuous variables between PBC patients and HCs. The Wilcoxon matched-pairs signed rank test was performed to compare the differences between TCR $\gamma\delta^{\text{high}}$ and TCR $\gamma\delta^{\text{low}}$ cells. The Kruskal–Wallis tests was performed to compare the differences among treatment-naïve patients, nonresponders and responders. The chi-squared test was applied to compare the categorical variables between PBC patients and HCs. A two-sided P value < 0.05 was considered significant. The analyses were conducted by using SPSS statistics version 26.

Results

Demographic and clinical characteristics of the subjects

The demographic and clinical characteristics of PBC patients and HCs are shown in Table 1. A total of 74 PBC patients and 74 age- and sex-matched HCs were enrolled in the study. Significant differences were observed in serum levels of alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), transaminase, albumin (ALB), total bilirubin (TBIL) and platelet count (PLT) between PBC patients and HCs. Among PBC patients, 12 were treatment-naïve, and 33 (44.6%) responded to UDCA therapy according to Paris I (for cirrhotic PBC patients) and Paris II criteria (for noncirrhotic PBC patients).

Table 1
Demographic and clinical characteristics of the subjects.

Characteristics	PBC (n = 74)	HCS (n = 74)	P value
Age, years	57 (48–66)	54 (48–62)	0.052
Female gender, n (%)	67 (90.5)	68 (91.2)	1.000
ALP, U/L	144 (99–236)	70 (55–83)	< 0.001
GGT, U/L	67 (36–140)	14 (13–18)	< 0.001
ALT, U/L	29 (18–44)	15 (13–20)	< 0.001
AST, U/L	33 (27–50)	20 (17–22)	< 0.001
ALB, g/L	40.3 (34.2–43.7)	42.7 (40.9–44.8)	0.001
TBIL, μ mol/L	17.6 (11.7–26.3)	12.6 (11.0–15.4)	< 0.001
PLT, 10^9 /L	168 (90–216)	235 (191–262)	< 0.001
IgG, mg/dl	1490 (1270–1730)	/	/
IgM, mg/dl	227 (122–355)	/	/
AMA-M ₂ (+), n (%)	64 (86.5)	/	/
Cirrhosis, n (%)	28 (37.8)	/	/
Treatment-naive, n (%)	12 (16.2)	/	/
UDCA responders [#] , n (%)	33 (44.6)	/	/
P values refer to comparisons between PBC patients and healthy controls.			
[#] Paris I and Paris II criteria were performed in cirrhotic and non-cirrhotic PBC patients to identify UDCA responders, respectively.			
PBC, primary biliary cholangitis; HCs, healthy controls; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; TBIL, total bilirubin; PLT, platelet count; IgG, immunoglobulin G; IgM, immunoglobulin M; AMA-M2, anti-mitochondrial M2 antibody; UDCA, ursodeoxycholic acid.			

Circulating TCR $\gamma\delta$ ^{low} cells were significantly decreased in PBC patients compared with HCs

$\gamma\delta$ T cells were defined as CD3 + TCR $\gamma\delta$ + cells in the study. The representative flow cytometric analysis demonstrated two distinct subsets based on the CD3/ $\gamma\delta$ TCR complex: the TCR $\gamma\delta$ ^{high} and TCR $\gamma\delta$ ^{low} subsets (Fig. 1a). The frequency of circulating TCR $\gamma\delta$ ^{low} cells among CD3 + cells was significantly higher than that of TCR $\gamma\delta$ ^{high} cells among CD3 + cells both in PBC patients (median 2.75% vs. 0.94%) and HCs (median 5.84% vs. 0.88%) (both $p < 0.001$).

The frequency and absolute number of circulating TCR $\gamma\delta^{\text{low}}$ cells were significantly decreased in PBC patients (n = 74) compared with HCs (n = 74) ($p < 0.001$), but the frequency and number of circulating TCR $\gamma\delta^{\text{high}}$ cells were similar between the two groups ($p > 0.05$) (Fig. 1b).

The frequency of circulating TCR $\gamma\delta^{\text{low}}$ cells was correlated with disease severity and UDCA response

We found a significant positive correlation between the frequency of circulating TCR $\gamma\delta^{\text{low}}$ cells and the serum level of PLT ($p = 0.001$, $r = 0.396$), but no correlations were found between the frequency of circulating TCR $\gamma\delta^{\text{low}}$ cells and the levels of TBIL, ALP or aspartate aminotransferase (AST) (Fig. 1c). Furthermore, the frequency and absolute number of circulating TCR $\gamma\delta^{\text{low}}$ cells were significantly decreased in cirrhotic PBC patients (n = 28) compared with noncirrhotic PBC patients (n = 46) (Fig. 1d).

Additionally, subgroup analysis showed that the frequency and absolute number of circulating TCR $\gamma\delta^{\text{low}}$ cells were the lowest in UDCA treatment-naïve PBC patients, followed by nonresponders and responders. A significantly decreased frequency and number of circulating TCR $\gamma\delta^{\text{low}}$ cells were detected in treatment-naïve patients (n = 12) and UDCA nonresponders (n = 29) compared with UDCA responders (n = 33) (Fig. 1e, $p < 0.05$).

The proportion of V δ 2 T cells in the circulating TCR $\gamma\delta^{\text{low}}$ subset was decreased in PBC patients

We further detected the expression of V δ 1 and V δ 2 in the TCR $\gamma\delta^{\text{high}}$ and TCR $\gamma\delta^{\text{low}}$ subsets (Fig. 2a). Approximately 75.1% of TCR $\gamma\delta^{\text{high}}$ cells were V δ 1 T cells and 19.3% were V δ 1⁻V δ 2⁻ T cells in HCs. The proportion of V δ 1 T cells in the TCR $\gamma\delta^{\text{high}}$ subset was not different between HCs and PBC patients ($p > 0.05$, Fig. 2b).

In contrast, the majority of TCR $\gamma\delta^{\text{low}}$ cells were V δ 2 T cells in HCs. The proportion of V δ 2 T cells in the TCR $\gamma\delta^{\text{low}}$ subset was decreased (89.4% vs. 97.4%), and the proportion of V δ 1⁻V δ 2⁻ T cells was increased (7.3% vs. 2.1%) in PBC patients compared with HCs ($p < 0.001$, Fig. 2c).

Circulating TCR $\gamma\delta^{\text{low}}$ cells exhibited similar apoptotic and proliferative phenotypes and enhanced CXCR6 expression in PBC patients

To explain the reduction in TCR $\gamma\delta^{\text{low}}$ cells in PBC patients, we assessed the apoptosis, proliferation, and expression of liver-homing chemokine receptors of $\gamma\delta$ T cells (Fig. 3a). No difference was observed in the expression of annexin-V (AV) and Ki67 in either circulating TCR $\gamma\delta^{\text{high}}$ cells or TCR $\gamma\delta^{\text{low}}$ cells between HCs and PBC patients ($p > 0.05$, Fig. 3b and 3c).

Compared with TCR $\gamma\delta^{\text{high}}$ cells, the expression of liver-homing chemokine receptors, including CXCR6 and CXCR3, was significantly higher in TCR $\gamma\delta^{\text{low}}$ cells in both HCs and PBC patients (all $p < 0.001$). The expression of CXCR6 in TCR $\gamma\delta^{\text{high}}$ cells and TCR $\gamma\delta^{\text{low}}$ cells was significantly increased in PBC patients

compared with HCs ($p < 0.05$, Fig. 3d), but the expression of CXCR3 in TCR $\gamma\delta^{\text{high}}$ cells and TCR $\gamma\delta^{\text{low}}$ cells was comparable between the two groups ($p > 0.05$, Fig. 3e).

Circulating TCR $\gamma\delta^{\text{low}}$ cells demonstrated an activated phenotype and enhanced GZMB production in PBC patients

The expression of activation markers, including CD69, HLA-DR, and CD25, in circulating $\gamma\delta$ T cells was analyzed in PBC patients and HCs. Circulating TCR $\gamma\delta^{\text{high}}$ cells were more activated than TCR $\gamma\delta^{\text{low}}$ cells in both HCs and PBC patients. Both the circulating TCR $\gamma\delta^{\text{high}}$ cells and TCR $\gamma\delta^{\text{low}}$ cells in PBC were more activated than those in HCs, characterized by higher expression of CD69 and HLA-DR ($p < 0.05$, Fig. 4a).

In addition, circulating TCR $\gamma\delta^{\text{high}}$ cells produced higher GZMB but lower IFN- γ and TNF- α than TCR $\gamma\delta^{\text{low}}$ cells in both HCs and PBC patients. We found an increased GZMB production of TCR $\gamma\delta^{\text{low}}$ cells in PBC patients ($n = 28$) compared with HCs ($n = 30$) ($p < 0.05$, Fig. 4c). The production of IFN- γ and TNF- α by TCR $\gamma\delta^{\text{low}}$ cells in PBC patients was lower than that in HCs, but the results remained statistically insignificant ($p > 0.05$, Fig. 4c).

Discussion

In the present study, we analyzed the number, phenotype, and functional molecules of $\gamma\delta$ T cells in PBC patients and sex- and age-matched HCs by flow cytometric analysis. We found that the number and function of TCR $\gamma\delta^{\text{low}}$ cells were significantly altered in PBC patients compared with HCs, which might be involved in the pathogenesis of PBC.

We identified two distinct subsets of circulating $\gamma\delta$ T cells in PBC patients according to CD3/TCR δ complex expression: the TCR $\gamma\delta^{\text{high}}$ and TCR $\gamma\delta^{\text{low}}$ subsets. Approximately three-quarters of cells in the TCR $\gamma\delta^{\text{high}}$ subset were V δ 1 T cells, while the majority of the TCR $\gamma\delta^{\text{low}}$ subset was V δ 2 T cells in HCs, which was consistent with the results of a previous study[13]. Furthermore, these two subpopulations differed from each other in terms of the expression levels of chemokines, activation markers, and functional molecules. Compared with the TCR $\gamma\delta^{\text{high}}$ subset, the TCR $\gamma\delta^{\text{low}}$ subset expressed higher levels of liver-homing chemokine receptors, lower activation markers, lower GZMB, and higher IFN- γ and TNF- α .

The change in the quantity of circulating $\gamma\delta$ T cells in PBC patients remains controversial in previous studies [9–12]. Studies have found a significantly decreased frequency of circulating $\gamma\delta$ T cells in PBC patients compared with HCs[11, 12], whereas the other two studies showed a similar frequency of $\gamma\delta$ T cells between the two groups[9, 10]. This might be due to the small sample sizes and different disease stages. The number of patients in our study was more than twice that of the previous studies. We found that the frequency and the absolute number of $\gamma\delta$ T cells was significantly decreased in PBC patients compared with HCs, and the $\gamma\delta$ T cells that were decreased in number were the those in the circulating TCR $\gamma\delta^{\text{low}}$ subset. The relatively large sample size allowed us to further analyze the frequency of TCR $\gamma\delta^{\text{low}}$ cells in subgroups. The results showed that the frequency of TCR $\gamma\delta^{\text{low}}$ cells was negatively

correlated with disease severity and positively correlated with the UDCA response, indicating that TCR $\gamma\delta^{\text{low}}$ cells might be a potential marker of disease progression and treatment response.

To explore the possible reasons for the reduction in TCR $\gamma\delta^{\text{low}}$ cells in PBC patients, we further detected the apoptotic and proliferative phenotype and the expression of liver-homing chemokine receptors on $\gamma\delta$ T cells. We found that circulating TCR $\gamma\delta^{\text{low}}$ cells exhibited a similar apoptotic and proliferative phenotype but enhanced liver-homing chemokine receptor (CXCR6) expression in PBC patients compared with HCs. Therefore, we postulated that the reduction in TCR $\gamma\delta^{\text{low}}$ cells might be caused by redistribution to the liver. In line with this hypothesis, studies found an increased number and frequency of hepatic $\gamma\delta$ T cells in autoimmune liver diseases and chronic liver diseases compared with HCs[9, 16, 17]. However, a recent study demonstrated a decreased frequency of hepatic $\gamma\delta$ T cells in 13 PBC and other chronic liver diseases patients compared with that in patients with healthy livers. Of note, all 13 of these PBC patients had end-stage liver disease, which might be an explanation for the opposite results. Future studies with larger sample sizes and different disease stages are needed to explore the quantity and function of $\gamma\delta$ T cells and their subpopulations in PBC livers.

Generally, the CXCR6-CXCL16 axis is considered to promote inflammation and disease progression in various liver diseases, including fibrosis[18], hepatocellular carcinoma[19], acute liver injury[20, 21], and nonalcoholic fatty liver disease[22]. Our results showed that approximately one-third of circulating TCR $\gamma\delta^{\text{low}}$ cells expressed CXCR6, and the proportion was even higher in PBC patients than in HCs. Consistent with our results, a recent study using single-cell RNA sequencing found that CXCR6 is preferentially expressed by hepatic $\gamma\delta$ T cells[17]. Taken together, this evidence suggests that $\gamma\delta$ T cells might be involved in liver injury through the CXCR6-CXCL16 axis.

The function of $\gamma\delta$ T cells in PBC has not been well elucidated. Human $\gamma\delta$ T cells display antiviral, proinflammatory or cytotoxic abilities in different liver and autoimmune diseases through the production of various cytokines, mainly IFN- γ , TNF- α , and GZMB[4, 5, 8, 23]. In this study, we analyzed the expression of activation markers and functional molecules of both the TCR $\gamma\delta^{\text{high}}$ and TCR $\gamma\delta^{\text{low}}$ subsets in PBC patients and HCs. We demonstrated that the circulating TCR $\gamma\delta^{\text{low}}$ subset exhibited an activated phenotype and enhanced cytotoxicity characterized by increased GZMB production in PBC patients, suggesting that the TCR $\gamma\delta^{\text{low}}$ subset might cause liver damage through GZMB production. Consistent with our results, Ferri S et al. found that $\gamma\delta$ T cells produced higher GZMB in 47 patients with autoimmune hepatitis than in 28 HCs, and the production of GZMB was correlated with biochemistry variables of liver damage[8]. In addition, another study demonstrated that circulating V δ 2 T cell function shifted from antiviral toward cytotoxicity after HCV infection, which is characterized by lower IFN- γ but higher GZMB produced by V δ 2 T cells[24]. Furthermore, the percentage of GZMB + V δ 2 T cells positively correlated with serum ALT levels in HCV-infected patients[24]. All these results showed that $\gamma\delta$ T cells might induce liver injury through enhanced GZMB production.

This study has several limitations. First, we failed to analyze the number and function of $\gamma\delta$ T cells in the liver by immunohistology, and further relevant research on hepatic $\gamma\delta$ T cells by new strategies, such as

RNA scope, is needed. Second, this is a cross-sectional study that enrolled patients with different disease stages and treatment responses. Long-term follow-up of the level of $\gamma\delta$ T cells in the same cohort before and after UDCA treatment will provide more value.

In summary, we identified two distinct functional subsets of circulating $\gamma\delta$ T cells according to the CD3/TCR $\gamma\delta$ complex in PBC patients. The TCR $\gamma\delta^{\text{low}}$ subset was significantly decreased in PBC patients and correlated with disease severity and the UDCA response, and this subset may play a crucial role in liver injury through increased CXCR6 expression and GZMB production.

Declarations

Funding This work is funded by National Natural Science Foundation of China (82000533).

Conflicts of interest The authors declare that they have no conflict of interest.

Ethics approval All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. The ethics committee of the Beijing Friendship Hospital, Capital Medical University reviewed and approved the study.

Consent to participate Written informed consent was obtained from all subjects enrolled in the study.

Consent for publication Written informed consent for publication was obtained from all participants.

Availability of data and material Not applicable.

Code availability Not applicable.

Authors' contributions Professors Jidong Jia and Dong Zhang designed, revised and finalized the manuscript; Doctor Sha Chen performed the experiment, analyzed the data and drafted the manuscript; Doctors Tingting Lv, Chunpan Zhang, Hua Jin, Mingyang Li, Guangyong Sun and Dan Tian participated in performing and guiding the experiment; Doctors Shuxiang Li, Weijia Duan, Shan Shan, and Hong Ma collected the blood sample and clinical data; Professors Guangyong, Sun, Xiaojuan Ou, and Hong You critically reviewed and revised the manuscript. All authors read and approved the final manuscript.

References

1. Lv T, Chen S, Li M, et al. Regional variation and temporal trend of primary biliary cholangitis epidemiology: A systematic review and meta-analysis. *J Gastroenterol Hepatol*. 2020;36:1423–34.
2. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: The diagnosis and management of patients with primary biliary cholangitis. *J Hepatol*. 2017;67:145–72.
3. Gao B, Jeong WI, Tian Z. Liver: An organ with predominant innate immunity. *Hepatology*. 2008;47:729–36.

4. Lawand M, Dechanet-Merville J, Dieu-Nosjean MC. Key Features of Gamma-Delta T-Cell Subsets in Human Diseases and Their Immunotherapeutic Implications. *Front Immunol.* 2017;8:761.
5. Rajoriya N, Fergusson JR, Leithead JA, et al. Gamma Delta T-lymphocytes in Hepatitis C and Chronic Liver Disease. *Frontiers in Immunology* 2014;5.
6. Vantourout P, Hayday A. Six-of-the-best: unique contributions of gammadelta T cells to immunology. *Nat Rev Immunol.* 2013;13:88–100.
7. Kenna T, Golden-Mason L, Norris S, et al. Distinct subpopulations of gamma delta T cells are present in normal and tumor-bearing human liver. *Clin Immunol.* 2004;113:56–63.
8. Ferri S, Longhi MS, De Molo C, et al. A multifaceted imbalance of T cells with regulatory function characterizes type 1 autoimmune hepatitis. *Hepatology.* 2010;52:999–1007.
9. Martins EB, Graham AK, Chapman RW, et al. Elevation of gamma delta T lymphocytes in peripheral blood and livers of patients with primary sclerosing cholangitis and other autoimmune liver diseases. *Hepatology.* 1996;23:988–93.
10. Hua F, Wang L, Rong X, et al. Elevation of Vdelta1 T cells in peripheral blood and livers of patients with primary biliary cholangitis. *Clin Exp Immunol.* 2016;186:347–55.
11. Niehues T, Gulwani-Akolkar B, Goldman IS, et al. Marked gamma delta T-cell decrease in peripheral blood of patients with primary biliary cirrhosis (PBC). *Autoimmunity.* 1994;18:267–73.
12. Jang JS, Juran BD, Cunningham KY, et al. Single-cell mass cytometry on peripheral blood identifies immune cell subsets associated with primary biliary cholangitis. *Sci Rep.* 2020;10:12584.
13. Yokobori N, Schierloh P, Geffner L, et al. CD3 expression distinguishes two gammadeltaT cell receptor subsets with different phenotype and effector function in tuberculous pleurisy. *Clin Exp Immunol.* 2009;157:385–94.
14. Venken K, Jacques P, Mortier C, et al. RORgammat inhibition selectively targets IL-17 producing iNKT and gammadelta-T cells enriched in Spondyloarthritis patients. *Nat Commun.* 2019;10:9.
15. Lindor KD, Bowlus CL, Boyer J, et al. Primary Biliary Cholangitis: 2018 Practice Guidance from the American Association for the Study of Liver Diseases. *Hepatology.* 2019;69:394–419.
16. Kanayama K, Morise K, Nagura H. Immunohistochemical study of T cell receptor gamma delta cells in chronic liver disease. *Am J Gastroenterol.* 1992;87:1018–22.
17. Zhao J, Zhang S, Liu Y, et al. Single-cell RNA sequencing reveals the heterogeneity of liver-resident immune cells in human. *Cell Discov.* 2020;6:22.
18. Wehr A, Baeck C, Heymann F, et al. Chemokine receptor CXCR6-dependent hepatic NK T Cell accumulation promotes inflammation and liver fibrosis. *J Immunol.* 2013;190:5226–36.
19. Gao Q, Zhao YJ, Wang XY, et al. CXCR6 upregulation contributes to a proinflammatory tumor microenvironment that drives metastasis and poor patient outcomes in hepatocellular carcinoma. *Cancer Res.* 2012;72:3546–56.
20. Wehr A, Baeck C, Ulmer F, et al. Pharmacological inhibition of the chemokine CXCL16 diminishes liver macrophage infiltration and steatohepatitis in chronic hepatic injury. *PLoS One.* 2014;9:e112327.

21. Wang H, Shao Y, Zhang S, et al. CXCL16 deficiency attenuates acetaminophen-induced hepatotoxicity through decreasing hepatic oxidative stress and inflammation in mice. *Acta Biochim Biophys Sin (Shanghai)*. 2017;49:541–9.
22. Ma KL, Wu Y, Zhang Y, et al. Activation of the CXCL16/CXCR6 pathway promotes lipid deposition in fatty livers of apolipoprotein E knockout mice and HepG2 cells. *Am J Transl Res*. 2018;10:1802–16.
23. O'Brien RL, Born WK. Two functionally distinct subsets of IL-17 producing gammadelta T cells. *Immunol Rev*. 2020;298:10–24.
24. Yin W, Tong S, Zhang Q, et al. Functional dichotomy of Vdelta2 gammadelta T cells in chronic hepatitis C virus infections: role in cytotoxicity but not for IFN-gamma production. *Sci Rep*. 2016;6:26296.

Figures

Figure 1

Decreased frequency and number of circulating TCR $\gamma\delta$ low cells in PBC patients. (a) Representative flow cytometric analysis of circulating $\gamma\delta$ T cells in HCs and PBC patients. (b) Decreased frequencies (left) and numbers (right) of circulating TCR $\gamma\delta$ low cells were detected in PBC patients (n=74) compared with HCs (n=74). (c) The frequency of circulating TCR $\gamma\delta$ low cells was positively correlated with PLT but not correlated with the levels of TBIL, ALP or AST (n = 74). (d) Decreased frequencies (left) and numbers (right) of circulating TCR $\gamma\delta$ low cells were detected in cirrhotic PBC patients (n=28) compared with noncirrhotic PBC patients (n=46). (e) Decreased frequencies (left) and numbers (right) of circulating

TCR $\gamma\delta$ low cells were detected in treatment-naïve PBC patients (n=12) and UDCA nonresponders (n=29) compared with UDCA responders (n=33). Data are presented as medians with interquartile ranges. *P < 0.05, **P < 0.01, ***P < 0.001, n.s. P > 0.05. PBC, primary biliary cholangitis; HCs, healthy controls; PLT, platelet count; TBIL, total bilirubin; ALP, alkaline phosphatase; AST, aspartate aminotransferase; UDCA, ursodeoxycholic acid.

Figure 2

The altered proportion of V δ 1 and V δ 2 T cells in the circulating TCR $\gamma\delta$ high and TCR $\gamma\delta$ low subsets. (a) Representative dot plot of V δ 1 and V δ 2 expression in TCR $\gamma\delta$ high cells and TCR $\gamma\delta$ low cells in HCs and PBC patients. (b) The proportions of V δ 1 and V δ 2 T cells in the circulating TCR $\gamma\delta$ high subset were similar between HCs (n=20) and PBC patients (n=20). (c) A decreased proportion of V δ 2 T cells and an increased proportion of V δ 1 T and V δ 1- V δ 2- T cells in circulating TCR $\gamma\delta$ low cells were detected in PBC patients (n=20) compared with HCs (n=20). Data are presented as medians with interquartile ranges. *P < 0.05, **P < 0.01, ***P < 0.001, n.s. P > 0.05. HCs, healthy controls; PBC, primary biliary cholangitis.

Figure 3

Alterations in apoptosis, proliferation, and the expression of liver-homing chemokine receptors in circulating TCR $\gamma\delta^{\text{high}}$ cells and TCR $\gamma\delta^{\text{low}}$ cells. (a) Representative flow cytometric analysis of AV, Ki67, CXCR6 and CXCR3 expression in circulating TCR $\gamma\delta^{\text{low}}$ cells in HCs and PBC patients. (b) No difference was observed in the expression of AV in either TCR $\gamma\delta^{\text{high}}$ cells or TCR $\gamma\delta^{\text{low}}$ cells between HCs (n=32) and PBC patients (n=28). (c) No difference was observed in the expression of Ki67 in either TCR $\gamma\delta^{\text{high}}$

cells or TCR $\gamma\delta$ low cells between HCs (n=24) and PBC patients (n=24). (d) Increased expression of CXCR6 in both circulating TCR $\gamma\delta$ high cells and TCR $\gamma\delta$ low cells was detected in PBC patients (n=49) compared with HCs (n=42). (e) No difference was observed in the expression of CXCR3 in either TCR $\gamma\delta$ high cells or TCR $\gamma\delta$ low cells between HCs (n=44) and PBC patients (n=47). Data are presented as medians with interquartile ranges. *P < 0.05, **P < 0.01, ***P < 0.001, n.s. P > 0.05. AV, Annexin V; HCs, healthy controls; PBC, primary biliary cholangitis.

Figure 4

Enhanced activation markers and altered cytokine production of circulating TCR $\gamma\delta$ ^{high} cells and TCR $\gamma\delta$ ^{low} cells. (a) Increased expression of CD69 (PBC: n=28; HC: n=28) and HLA-DR (PBC: n=32; HC: n=29) in both TCR $\gamma\delta$ ^{high} cells and TCR $\gamma\delta$ ^{low} cells was detected in PBC patients compared with HCs. No difference was observed in the expression of CD25 in TCR $\gamma\delta$ ^{high} cells and TCR $\gamma\delta$ ^{low} cells between HCs (n=28) and PBC patients (n=25). (b) Representative dot plot of cytokine production of TCR $\gamma\delta$ ^{low} cells stimulated by PMA-ionomycin. (c) Increased expression of GZMB in circulating TCR $\gamma\delta$ ^{low} cells was detected in PBC patients (n=28) compared with HCs (n=30) ($P < 0.05$). No difference was observed in the expression of IFN- γ (PBC: n=25; HC: n=26) or TNF- α (PBC: n=22; HC: n=20) in either TCR $\gamma\delta$ ^{high} cells or TCR $\gamma\delta$ ^{low} cells between PBC patients and HCs. Data are presented as medians with interquartile ranges. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, n.s. $P > 0.05$. GZMB, granzyme B; HCs, healthy controls; PBC, primary biliary cholangitis.