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

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Research Article
Abstract

Background & Aims

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

Methods

We analyzed the number, phenotypes, and functional molecules of γδ 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 γδ T cells according to the CD3/TCRγδ complex: the TCRγδhigh and TCRγδlow subsets. Approximately three-quarters of cells in the TCRγδhigh subset were Vδ1 T cells, while Vδ2 T cells were enriched in the TCRγδlow subset in HCs. The frequency and absolute number of circulating TCRγδlow cells was significantly decreased in PBC patients compared with HCs (p < 0.001). Furthermore, the frequency of TCRγδlow cells was negatively correlated with disease severity and positively correlated with the ursodeoxycholic acid response. TCRγδlow 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 TCRγδhigh and TCRγδlow 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 TCRγδlow cells in PBC patients compared with HCs.

Conclusion

The TCRγδlow 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 (γδ) T cells are a unique population of unconventional T cells with γ and δ glycoprotein chains. In humans, γδ 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 γδ T cells are closely involved in the development of viral hepatitis and autoimmune liver diseases[5, 8, 9]. However, the frequency of γδ T cells in PBC remains controversial in previous studies with small sample sizes[9–12]. The function of γδ T cells in PBC also needs to be further explored.

Additionally, most works on human γδ T cells have focused on Vδ1 and Vδ2 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 γδ T cells, called the TCRγδ^high and TCRγδ^low subsets, in patients with tuberculous pleurisy according to CD3/TCRγδ complex expression[13]. In line with this, a recent study found different expression levels of transcription factors, including PLZF and RORγt, between human circulating TCRγδ^high and TCRγδ^low cells[14], indicating that the two distinct subsets might have different phenotypes and functions. However, the role of the TCRγδ^high and TCRγδ^low subsets in PBC remains unclear.

Therefore, our study aimed to explore the number, phenotype, and functional molecules of γδ T cells in PBC, with a special focus on the TCRγδ^high and TCRγδ^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 γδ T cells**

Flow cytometry was performed using the following antibodies: anti-CD3-Brilliant Violet 711, anti-TCRγδ-PE, anti-TCRδ2-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-TCRVδ1-PE/Cy7 was obtained from Thermo Scientific (Waltham, MA, USA).

Flow cytometry was performed using a FACSria 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 γδ 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 μg/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 TCRγδ<sup>high</sup> and TCRγδ<sup>low</sup> 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, µmol/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-M2 (+), 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.

Circulating TCRγδ low cells were significantly decreased in PBC patients compared with HCs

γδ T cells were defined as CD3 + TCRγδ + cells in the study. The representative flow cytometric analysis demonstrated two distinct subsets based on the CD3/γδTCR complex: the TCRγδ high and TCRγδ low subsets (Fig. 1a). The frequency of circulating TCRγδ low cells among CD3 + cells was significantly higher than that of TCRγδ 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 TCRγδ\textsubscript{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 TCRγδ\textsubscript{high} cells were similar between the two groups (p > 0.05) (Fig. 1b).

**The frequency of circulating TCRγδ\textsubscript{low} cells was correlated with disease severity and UDCA response**

We found a significant positive correlation between the frequency of circulating TCRγδ\textsubscript{low} cells and the serum level of PLT (p = 0.001, r = 0.396), but no correlations were found between the frequency of circulating TCRγδ\textsubscript{low} cells and the levels of TBIL, ALP or aspartate aminotransferase (AST) (Fig. 1c). Furthermore, the frequency and absolute number of circulating TCRγδ\textsubscript{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 TCRγδ\textsubscript{low} cells were the lowest in UDCA treatment-naïve PBC patients, followed by nonresponders and responders. A significantly decreased frequency and number of circulating TCRγδ\textsubscript{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 Vδ2 T cells in the circulating TCRγδ\textsubscript{low} subset was decreased in PBC patients**

We further detected the expression of Vδ1 and Vδ2 in the TCRγδ\textsubscript{high} and TCRγδ\textsubscript{low} subsets (Fig. 2a). Approximately 75.1% of TCRγδ\textsubscript{high} cells were Vδ1 T cells and 19.3% were Vδ1-Vδ2\textsuperscript{−} T cells in HCs. The proportion of Vδ1 T cells in the TCRγδ\textsubscript{high} subset was not different between HCs and PBC patients (p > 0.05, Fig. 2b).

In contrast, the majority of TCRγδ\textsubscript{low} cells were Vδ2 T cells in HCs. The proportion of Vδ2 T cells in the TCRγδ\textsubscript{low} subset was decreased (89.4% vs. 97.4%), and the proportion of Vδ1-Vδ2\textsuperscript{−} T cells was increased (7.3% vs. 2.1%) in PBC patients compared with HCs (p < 0.001, Fig. 2c).

**Circulating TCRγδ\textsubscript{low} cells exhibited similar apoptotic and proliferative phenotypes and enhanced CXCR6 expression in PBC patients**

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

Compared with TCRγδ\textsubscript{high} cells, the expression of liver-homing chemokine receptors, including CXCR6 and CXCR3, was significantly higher in TCRγδ\textsubscript{low} cells in both HCs and PBC patients (all p < 0.001). The expression of CXCR6 in TCRγδ\textsubscript{high} cells and TCRγδ\textsubscript{low} cells was significantly increased in PBC patients.
compared with HCs ($p < 0.05$, Fig. 3d), but the expression of CXCR3 in TCRγδ$_{\text{high}}$ cells and TCRγδ$_{\text{low}}$ cells was comparable between the two groups ($p > 0.05$, Fig. 3e).

**Circulating TCRγδ$_{\text{low}}$ 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 γδ T cells was analyzed in PBC patients and HCs. Circulating TCRγδ$_{\text{high}}$ cells were more activated than TCRγδ$_{\text{low}}$ cells in both HCs and PBC patients. Both the circulating TCRγδ$_{\text{high}}$ cells and TCRγδ$_{\text{low}}$ 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 TCRγδ$_{\text{high}}$ cells produced higher GZMB but lower IFN-γ and TNF-α than TCRγδ$_{\text{low}}$ cells in both HCs and PBC patients. We found an increased GZMB production of TCRγδ$_{\text{low}}$ 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 TCRγδ$_{\text{low}}$ 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 γδ T cells in PBC patients and sex- and age-matched HCs by flow cytometric analysis. We found that the number and function of TCRγδ$_{\text{low}}$ 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 γδ T cells in PBC patients according to CD3/TCRγδ complex expression: the TCRγδ$_{\text{high}}$ and TCRγδ$_{\text{low}}$ subsets. Approximately three-quarters of cells in the TCRγδ$_{\text{high}}$ subset were Vδ1 T cells, while the majority of the TCRγδ$_{\text{low}}$ subset was Vδ2 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 TCRγδ$_{\text{high}}$ subset, the TCRγδ$_{\text{low}}$ 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 γδ T cells in PBC patients remains controversial in previous studies [9–12]. Studies have found a significantly decreased frequency of circulating γδ T cells in PBC patients compared with HCs[11, 12], whereas the other two studies showed a similar frequency of γδ 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 γδ T cells was significantly decreased in PBC patients compared with HCs, and the γδ T cells that were decreased in number were the those in the circulating TCRγδ$_{\text{low}}$ subset. The relatively large sample size allowed us to further analyze the frequency of TCRγδ$_{\text{low}}$ cells in subgroups. The results showed that the frequency of TCRγδ$_{\text{low}}$ cells was negatively
correlated with disease severity and positively correlated with the UDCA response, indicating that TCRγδ^low^ cells might be a potential marker of disease progression and treatment response.

To explore the possible reasons for the reduction in TCRγδ^low^ cells in PBC patients, we further detected the apoptotic and proliferative phenotype and the expression of liver-homing chemokine receptors on γδ T cells. We found that circulating TCRγδ^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 TCRγδ^low^ cells might be caused by redistribution to the liver. In line with this hypothesis, studies found an increased number and frequency of hepatic γδ 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 γδ 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 γδ 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 TCRγδ^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 γδ T cells[17]. Taken together, this evidence suggests that γδ T cells might be involved in liver injury through the CXCR6-CXCL16 axis.

The function of γδ T cells in PBC has not been well elucidated. Human γδ 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 TCRγδ^high^ and TCRγδ^low^ subsets in PBC patients and HCs. We demonstrated that the circulating TCRγδ^low^ subset exhibited an activated phenotype and enhanced cytotoxicity characterized by increased GZMB production in PBC patients, suggesting that the TCRγδ^low^ subset might cause liver damage through GZMB production. Consistent with our results, Ferri S et al. found that γδ 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 Vδ2 T cell function shifted from antiviral toward cytotoxicity after HCV infection, which is characterized by lower IFN-γ but higher GZMB produced by Vδ2 T cells[24]. Furthermore, the percentage of GZMB^+^ Vδ2 T cells positively correlated with serum ALT levels in HCV-infected patients[24]. All these results showed that γδ 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 γδ T cells in the liver by immunohistology, and further relevant research on hepatic γδ 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 γδ 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 γδ T cells according to the CD3/TCRγδ complex in PBC patients. The TCRγδ\textsuperscript{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.

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Figures
Figure 1

Decreased frequency and number of circulating TCRγδlow cells in PBC patients. (a) Representative flow cytometric analysis of circulating γδ T cells in HCs and PBC patients. (b) Decreased frequencies (left) and numbers (right) of circulating TCRγδlow cells were detected in PBC patients (n=74) compared with HCs (n=74). (c) The frequency of circulating TCRγδlow 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 TCRγδlow 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...
TCRγδlow 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 Vδ1 and Vδ2 T cells in the circulating TCRγδhigh and TCRγδlow subsets. (a) Representative dot plot of Vδ1 and Vδ2 expression in TCRγδhigh cells and TCRγδlow cells in HCs and PBC patients. (b) The proportions of Vδ1 and Vδ2 T cells in the circulating TCRγδhigh subset were similar between HCs (n=20) and PBC patients (n=20). (c) A decreased proportion of Vδ2 T cells and an increased proportion of Vδ1 T and Vδ1- Vδ2- T cells in circulating TCRγδlow 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 TCRγδhigh cells and TCRγδlow cells. (a) Representative flow cytometric analysis of AV, Ki67, CXCR6 and CXCR3 expression in circulating TCRγδlow cells in HCs and PBC patients. (b) No difference was observed in the expression of AV in either TCRγδhigh cells or TCRγδlow cells between HCs (n=32) and PBC patients (n=28). (c) No difference was observed in the expression of Ki67 in either TCRγδhigh
cells or TCRγδlow cells between HCs (n=24) and PBC patients (n=24). (d) Increased expression of CXCR6 in both circulating TCRγδhigh cells and TCRγδlow 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 TCRγδhigh cells or TCRγδlow 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.
Enhanced activation markers and altered cytokine production of circulating TCRγδhigh cells and TCRγδlow cells. (a) Increased expression of CD69 (PBC: n=28; HC: n=28) and HLA-DR (PBC: n=32; HC: n=29) in both TCRγδhigh cells and TCRγδlow cells was detected in PBC patients compared with HCs. No difference was observed in the expression of CD25 in TCRγδhigh cells and TCRγδlow cells between HCs (n=28) and PBC patients (n=25). (b) Representative dot plot of cytokine production of TCRγδlow cells stimulated by PMA-ionomycin. (c) Increased expression of GZMB in circulating TCRγδlow 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 TCRγδhigh cells or TCRγδlow 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.