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ORIGINAL ARTICLE

Higher frequency of circulating Vδ1 γδT cells in patients with advanced schistosomiasis

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Abstract

Gamma-delta (γδ) T cells are the bridge between natural and adaptive immunity. In the present study, peripheral blood was collected from 13 patients with advanced schistosomiasis (schistosomiasis group) and 13 uninfected people (control group) to investigate the γδ T cells and their subtypes in human schistosomiasis. Compared with the control group, the proportion of Vδ1 cells and $CD27^{+}V\delta1^{+}$ cells in the schistosomiasis group increased significantly, while $CD27^{-}$ cells and $CD27^{-}V\delta1^{-}$ cells decreased. Only the level of IL-17A differed between the groups, being significantly decreased in the schistosomiasis group. In the schistosomiasis group, there were no correlations between the liver fibrosis and subsets of γδ T cells, or the level of cytokines. Additionally, the level of IL-17A correlated positively with the proportion of $CD27^{-}V\delta1^{-}$ cells. Thus, there was a higher frequency of circulating Vδ1 γδT cells in patients with advanced schistosomiasis. The decreased IL-17A might be related to the reduction in $CD27^{-}V\delta1^{-}$ cell.

KEYWORDS
fibrosis, IL-17, *Schistosoma japonicum*, schistosomiasis, γδ T cell

1 | INTRODUCTION

Schistosomiasis affects more than 200 million people worldwide.[1] Previously, *Schistosoma japonicum* was endemic in China, especially in areas with many lakes, such as the 'Four Lakes Area' in Hubei province. Many patients with chronic schistosomiasis develop advanced schistosomiasis each year, despite not coming into contact with the cercariae for a long time. For more than 50 years, researchers worldwide have studied changes in the host's immune environment during the progress of infection. While the functions of helper T (Th) cells, cytotoxic T (Tc) cells and B cells are well known, the responses and functions of γδ T cells during schistosomiasis remain unclear.

γδ T cells are the first line of immune defence, playing an important role in cancer, autoimmunity and infectious diseases. They

possess strong antimicrobial activity against viral, bacterial and other pathogens. In addition to γδ T cell receptors (TCRs), γδ T cells express all T-lineage-specific genes, including those encoding cell surface receptors, signalling factors, cytokines and transcription factors. Their phenotypes and functions are similar to those of activated αβ T cells.[2] CD27 is a marker that can distinguish between the interferon-γ (IFN-γ) and interleukin-17 (IL-17) producing γδ T cell subsets in a mouse model[3]; however, it is unknown whether the marker is applicable in humans.

Human γδ T cells are divided into three main categories depending on the δ chain: Vδ1 T cells, Vδ2 T cells and Vδ3 T cells.[4] The Vδ1 chain-expressing cells are mainly present in the epithelium of the mucous membrane, where they are involved in maintaining the integrity of epithelial tissue when faced with damage, infection and transplantation.[5] Vδ1 T cells also appear in the peripheral blood; however, the mucosal and peripheral blood γδ T cells appear to be distinct populations.[6] Vδ1 T cells produce abundant IFN-γ and are thought to be an important source of IL-17.[7] Most circulating γδ T cells in healthy adults are Vδ2 chain-expressing T cells, with a 50-90% ratio. Vδ2 T cells are almost entirely Vγ9-expressing cells.[8] Vδ2 T cells produce IFN-γ, IL-17A and tumour necrosis factor-alpha (TNF-α) to promote inflammation and induce anti-infective immunity in different settings of infectious diseases.[9] Although the third class, Vδ3 T cells, represent only about 0.2% of circulating T cells, they are abundant in the liver and are increased in patients with leukaemia and in some patients with chronic viral infections. When activated, they can kill $CD1d^+$ target cells and induce Th1, Th2 and Th17 cells to release cytokines.[10]

Most studies have observed the characteristics and explored the functions of γδ T cells using mouse models, while few studies have investigated their function in humans infected with *S japonicum*.[11-13] In 2014, Schwartz et al[14] reported the similarities and differences in the characteristics of γδ T cells during *S hematobium* or *S mansoni* infections in humans. However, schistosomiasis caused by *S japonicum* is a much more serious disease than that caused by other *Schistosoma* species.[15] When the eggs are deposited in the liver, intestines and spleen of a patient, they can persist for a long time, leading to chronic injury and liver fibrosis. If the patient is subjected to repeated infection, even if treated, 5-10 years later they would develop advanced schistosomiasis, including cirrhosis ascites, portal hypertension and a significantly reduced quality of life.[16] Therefore, it is necessary to explore the details of the change of the immune phenotype and quantity of γδ T cells in patients with *S japonicum* infection.

In previous research, our team observed the characteristics of γδ T cells and related cytokines in a mouse model of *S japonicum* infection, especially the subset of γδ T cells that produced cytokines (IL-17/IFN-γ) in uninfected conditions, but expressed decreased levels as the infection progressed, with almost none being produced in the late stage.[17] We found that the Vγ2 subset of γδ T cells might play an important role in accelerating liver fibrosis by recruiting neutrophils during *S japonicum* infection. However, Hammerich et al[18] found that γδ T cells could protect the liver from excessive inflammation and fibrosis by inhibiting hepatic stellate cells (HSCs). Meanwhile, Markovits et al observed anti-fibrotic characteristics of $V\gamma9^+$ γδ T cells in systemic sclerosis,[19] which presents a paradox as to whether γδ T cells played a pro-fibrosis or anti-fibrosis role in the human liver; therefore, we were interested in defining the exact relationship between γδ T cells and liver fibrosis in human patients infected with *S japonicum*.

The present study aimed to investigate the immune status of a population with schistosomiasis and to verify that γδ T cells produce cytokines such as IL-17 during the progress of *S japonicum* infection. In addition, we explored which subtypes of γδ T cells function during infection, and the relationship between γδ T cells and the degree of liver fibrosis.

2 | METHODS

2.1 | Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, China (Permit Number: IPD 2017-006). Before sample collection, the survey and the procedure for sample collection were explained to the patients and the control subjects in the hospital. Each participant provided signed informed consent. All patients were treated without charge.

2.2 | Patients and sample collection

According to the 'Schistosomiasis Diagnostic Standard' (WS261-2006) provided by the Ministry of Health of the People's Republic of China, advanced schistosomiasis was defined as a patient with schistosomiasis causing liver fibrosis, portal hypertension symptoms, severe growth disorders or colon granuloma growth. Repeated or large-scale infection by *Schistosoma japonicum*, if not treated thoroughly and timely, a patient could develop advanced schistosomiasis, usually after 2-10 years of pathological development. Clinical symptoms of advanced schistosomiasis include abdominal fluid, spleen enlargement, high blood pressure, gastroesophageal varicose bleeding, large intestine granuloma lesions and severe growth retardation. People with advanced schistosomiasis were documented in the CDC (Center for Disease Control) surveillance system from the time they were first infected.

All candidates were from the Schistosomiasis control hospital of Qian-jiang City in Hubei province (China). Thirteen patients (aged 63 ± 9.63 years) with advanced schistosomiasis were selected as the schistosomiasis group, while 13 healthy controls (aged 57 ± 8.05 years) comprised the control group. All the recruited patients had no active infection. They tested negative for active eggs via the faecal miracidia hatching method. When the patients were in a stable condition, they all received praziquantel at the appropriate time. The healthy people in the control group were recruited from the physical examination centre in the same hospital. All candidates

completed forms agreeing to participate in the study. Peripheral blood samples were obtained from the two groups.

Peripheral blood from the candidates was collected into tubes with and without sodium heparin, separately. The serum samples were isolated after 2 hours of storage at room temperature. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Hypaque (TBD).

2.3 | Flow cytometry

Blood samples were collected in tubes containing sodium heparin and treated with NH_4Cl erythrocyte lysis buffer. The obtained single-cell suspensions were stained with directly conjugated antibodies (listed below) for 30 minutes at 4°C in the dark in phosphate-buffered saline/1% bovine serum albumin. Dead cells were excluded by adding Fixable viability dye eFluor 780 (1:1,000; eBioscience). All antibodies were purchased from Life Technologies (Gaithersburg), except for the antibodies against Vδ1, which were from Abcam (Cambridge), and the γδ TCR and CD27 antibodies, which were from eBioscience. The following antibodies were used in the γδ T cell phenotyping panel: CD45-eFluor 450 (1:100; clone HI30), Vδ1-fluorescein isothiocyanate (FITC) (1:200; clone TS8.2), γδ TCR-phycoerythrin (PE) (1:200; clone B1.1), CD27-Allophycocyanin (APC) (1:100; clone O323), CD4-APC-eFluor 780 (1:200; clone OKT4), CD19- APC-eFluor 780 (1:200; clone ICRF44), CD11b-APC-eFluor 780 (1:200; clone HIB19) and fixable viability dye eFluor 780. All experiments were performed using a CytoFLEX flow cytometer (Beckman Coulter, Indianapolis, IN, USA) using CytExpert software (Beckman Coulter). Data analyses used FlowJo Software version 9.7.1 (Becton Dickinson).

2.4 | Enzyme-linked immunosorbent assay (Elisa) and serum analysis

ELISA was performed according to the manufacturer's instructions to detect the serum levels of IL-4, IL-6, IL-10, IL-17A, IL-21, transforming growth factor-beta (TGF-β), IL-1β and IL-22. All ELISA kits were purchased from eBioscience (Invitrogen, Waltham, MA, USA).

The levels of type III procollagen (PC-III) and hyaluronic acid (HA) in the serum were assayed via a Chemiluminescent immunoassay by the Kindstar Global Department of Pathology.

2.5 | Quantitative real-time reverse transcription PCR

RNA was extracted from peripheral blood according to the manufacturer's instructions using an X-press Blood RNA kit (Omega Bio-Tek). Reverse transcriptase (RT) reactions for cDNA synthesis were performed using PrimeScript RT Master Mix (Takara). Relative mRNA expression levels were determined using quantitative real-time polymerase chain reaction (qPCR) with an SYBR Green I PCR Master Mix kit (Takara) on an ABI ViiATM7 machine (Applied Biosystems) according to the manufacturer's protocol. The primer sequences were IL-17A forward 5′-TCCCACGAAATCCAGGATGC-3′,

reverse 5′-GGATGTTCAGGTTGACCATCAC-3′; Glyceraldehyde-3-phosphate dehydrogenase (GAPDH; control) forward 5′-CCAAGGAGTAAGACCCCTGG-3′,

reverse 5′- TGGTTGAGCACAGGGTACTT-3′.

2.6 | Statistical analysis

The results were analysed using GraphPad Prism 5.0 (GraphPad Software, Inc,). All data are presented as mean ± standard deviation. The differences between two groups were analysed using an independent t-test or Welch's t-test. The correlation between groups was tested using Spearman's rank correlation. $P < .05$ was chosen as a sensitive level of significance.

3 | Results

3.1 | The proportion of subsets of total γδ T cells detected by flow cytometry

The results showed that, compared with those in the control group, the proportion of Vδ1 cells in the patients with advanced schistosomiasis increased significantly ($P = .0007$), with a significant increase in the proportion of $CD27^+V\delta1^+$ cells ($P = .0182$). We also observed a significant decrease in the ratio of $CD27^-$ cells ($P = .0055$), among which the proportion of $CD27^-V\delta1^-$ cells also decreased ($P < .0001$) (Figure 1A,B).

Among the total γδ T cells, the percentage of $V\delta1^+$ cells was 20.53 ± 3.05% in the control group and 52.69 ± 7.74% in the schistosomiasis group, while the percentage of $CD27^+V\delta1^+$ cells was 5.46 ± 3.79% in the control group, which increased to 20.20 ± 4.42% in the schistosomiasis group. By contrast, the percentage of $CD27^-$ cells was 85.54 ± 3.85% in the control group and 61.94 ± 6.70% in the schistosomiasis group, while the percentage of $CD27^-V\delta1^-$ cells was 68.18 ± 3.17% in the control group, which decreased to 29.46 ± 5.811% in the schistosomiasis group. All the results are shown as the mean ± SEM%.

3.2 | The level of cytokines in serum detected by ELISA

The results showed that there were no significant differences between the two groups for most of the cytokines (IL-4, IL-6, IL-10, IL-21, TGF-β, IL-1β, and IL-22); however, there was a significant decrease in the levels of IL-17A ($P = .013$), from 32.16 ± 2.55 pg/mL in the control group to 23.42 ± 1.99 pg/mL in the serum of patients with advanced schistosomiasis (Figure 1C).

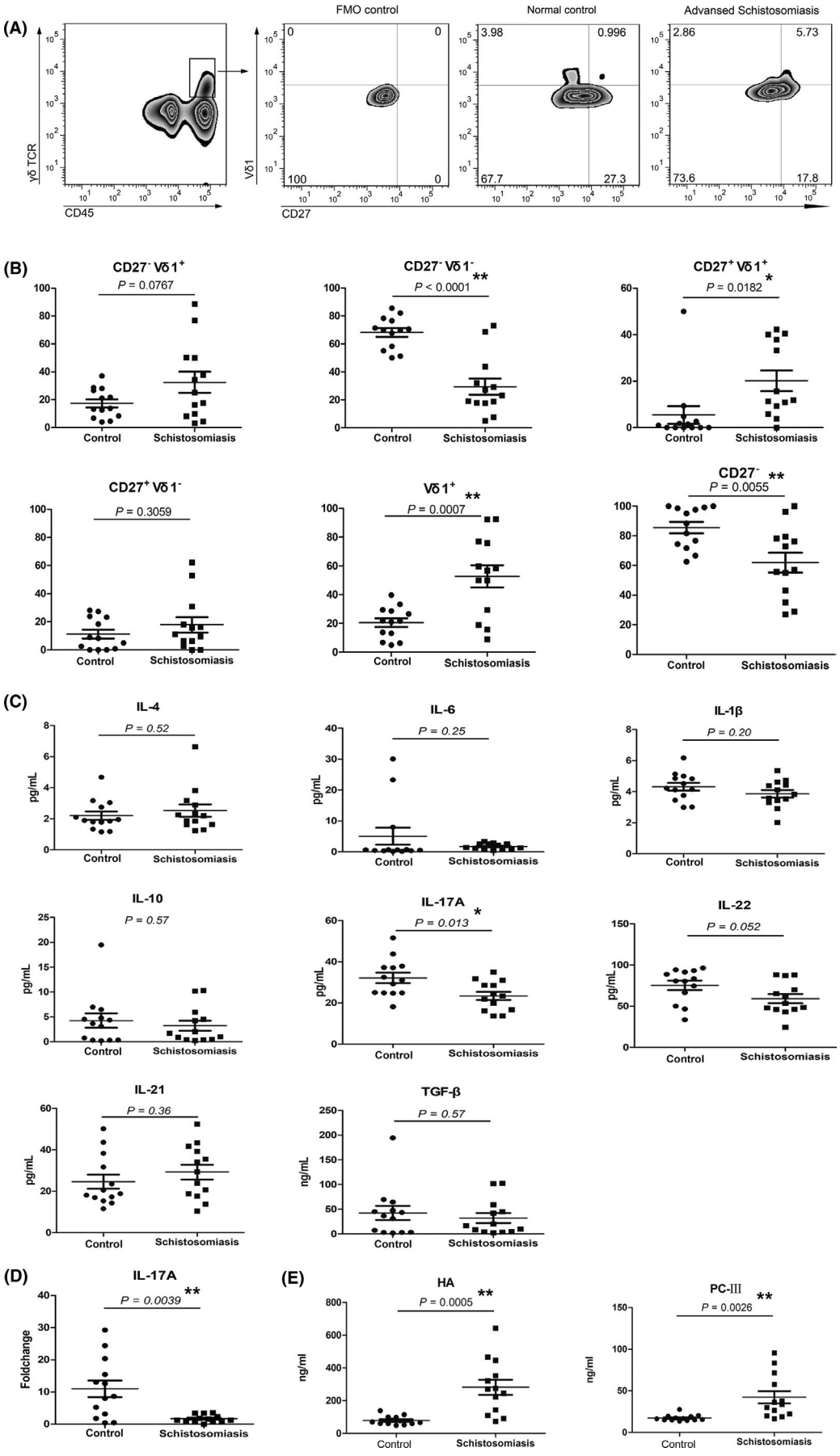
(A)
FMO control
Normal control
Advansed Schistosomiasis
γδ TCR
CD45
Vδ1
CD27
0
0
100
0
3.98
0.996
67.7
27.3
2.86
5.73
73.6
17.8
(B)
CD27⁻ Vδ1⁺
P = 0.0767
CD27⁻ Vδ1⁻
P < 0.0001 **
CD27⁺ Vδ1⁺
P = 0.0182 *
CD27⁺ Vδ1⁻
P = 0.3059
Vδ1⁺
P = 0.0007 **
CD27⁻ **
P = 0.0055
Control
Schistosomiasis
(C)
IL-4
P = 0.52
IL-6
P = 0.25
IL-1β
P = 0.20
IL-10
P = 0.57
IL-17A
P = 0.013 *
IL-22
P = 0.052
IL-21
P = 0.36
TGF-β
P = 0.57
pg/mL
ng/mL
(D)
IL-17A
P = 0.0039 **
Foldchange
(E)
HA
P = 0.0005 **
PC-III
P = 0.0026 **
ng/ml

FIGURE 1 Changes to the subsets of γδ T cells and the related cytokines in the serum from different groups. (A, B) Representative flow cytometry plots are shown to describe the changes in the proportions of subsets of γδ T cells in subjects with or without advanced schistosomiasis. Summary graphs showing the increase and decrease in subsets of γδ T cells as a percentage of total γδ T cells compared with those in healthy controls. (C) Differences in the cytokine profiles between the two groups. (D) The mRNA expression of *IL17A* from white cells of peripheral blood as assessed using qRT-PCR. Fold-change is the logarithmic ratio (log2 ratio) compared with the expression level in the control. (E) The level of type III procollagen (PC-III) and hyaluronic acid (HA) in the serum were tested using a chemiluminescent immunoassay (CLIA). Data are shown as the mean ± SE, and the *P* values are shown between the data in each column (by Student's t-test or with Welch's correction). *:$P < .05$. **:$P < .01$

3.3 | The level of cytokines from PBMCs tested by QRT-PCR

To check whether the level of cytokines from the PBMCs was similar to the trends shown in ELISA results, we tested the fold-change of mRNA from the same samples using qRT-PCR. The results showed that the relative mRNA level of *IL-17A* from the peripheral blood of the patients with advanced schistosomiasis was significantly reduced compared with that in the control group ($P = .0039$) (Figure 1D). By measuring the relative change of expression of *IL-17A* by qRT-PCR, we confirmed that the source of PBMCs was reduced compared with that in the healthy controls.

3.4 | The levels of HA and PC-III IN serum directly reflected the degree of hepatic fibrosis

The results showed a significant increase in HA and PC-III in the serum of the advanced schistosomiasis group compared with that in the healthy control group (Figure 1E). The HA level was 79.22 ± 7.41 ng/mL in the healthy controls, which increased to 281.70 ± 46.04 ng/mL in the schistosomiasis group ($P = .0005$). The level of PC-III was 17.30 ± 1.04 ng/mL in the healthy controls, which increased to 42.40 ± 7.28 ng/mL in the *S japonicum* group ($P = .0026$). The levels of HA and PC-III in serum directly reflected the degree of hepatic fibrosis.

3.5 | The relations between the Vδ1$^+$ cells, CD27$^+$Vδ1$^+$ cells and cytokines

The proportions of Vδ1$^+$ cells and CD27$^+$Vδ1$^+$ cells increased; therefore, we attempted to determine if there was a relationship between the two subsets and cytokines. The results showed that the levels of cytokines did not correlate positively with the proportion of Vδ1$^+$ cells (Figure 2A) or CD27$^+$Vδ1$^+$ cells (Figure 2B) in total γδ T cells. It was worth noting that the *P* values of IL-6 and IL-21 were .0607 and .0640 for the correlation with CD27$^+$Vδ1$^+$ cells, respectively, which were close to statistical significance.

3.6 | The proportions of subsets or cytokines did not correlate with liver fibrosis

In the group of patients with advanced schistosomiasis, neither the proportion of Vδ1 cells nor CD27$^-$ cells correlated significantly with liver fibrosis (Figure 3A). In addition, we detected the level of the cytokines in the serum and assessed their relationship with liver fibrosis. The results showed no significant correlation between IL-4, IL-6, IL-10, IL-17A, IL-21, TGF-β, IL-1β, or IL-22 and liver fibrosis (Figure 3B).

3.7 | Levels of IL-17A in serum correlated with the proportions of CD27$^-$ Vδ1$^-$ γδ T cells

The results showed that the level of IL-17A in the serum correlated positively with the proportion of CD27$^-$Vδ1$^-$ cells among total γδ T cells ($r = 0.6703$, $P = .0122$) (Figure 4). None of the other subtypes of γδ T cells correlated significantly with IL-17A levels.

4 | DISCUSSION

In mouse models, γδ T cells were mainly divided into Vγ1 and Vγ2 cells according to the different γ chains. In our previous study, compared with normal mice, the ratio of Vγ1 and Vγ2 cells did not change significantly in early or late infection of *S japonicum*.[17] In contrast, according to the different δ chains, in human peripheral blood, γδ T cells were mainly divided into Vδ1 and Vδ2 cells.[20] In the present study, there was a change in the percentage of Vδ1 and Vδ2 cells in patients with advanced schistosomiasis compared with that in the control group, that is, the proportion of Vδ1 cells increased, and the proportion of Vδ2 cells decreased. There might be two reasons for the increase in Vδ1 cells. The first was antigen stimulation, and the second was the relative decline of Vδ2 cells. Vδ1 cells are capable of proliferating in response to signals through TCRs and IL-15.[21] However, during the advanced stage of schistosomiasis, the immune environment is suppressed. In the peripheral blood of healthy adults, the proportion of the Vδ2 subset among total γδ T cells was greater than 70%. This subtype plays an important anti-infection and anti-tumour role. Therefore, we believe that the second explanation is more likely.

In our previous work, we demonstrated that the γδ T cells lost their ability to secret IL-17A as schistosomiasis progressed.[17] The results of the present study partially confirmed this: Compared with that in the healthy controls, the level of the IL-17A in serum was significantly lower in the patients with advanced schistosomiasis. Chen et al[22] found that γδ T cells are the main IL-17-producing cells in PBMCs and that IL-17 contributes to granulomatous inflammatory and fibrosing reactions during *S japonica* infection. Wang et al[23]

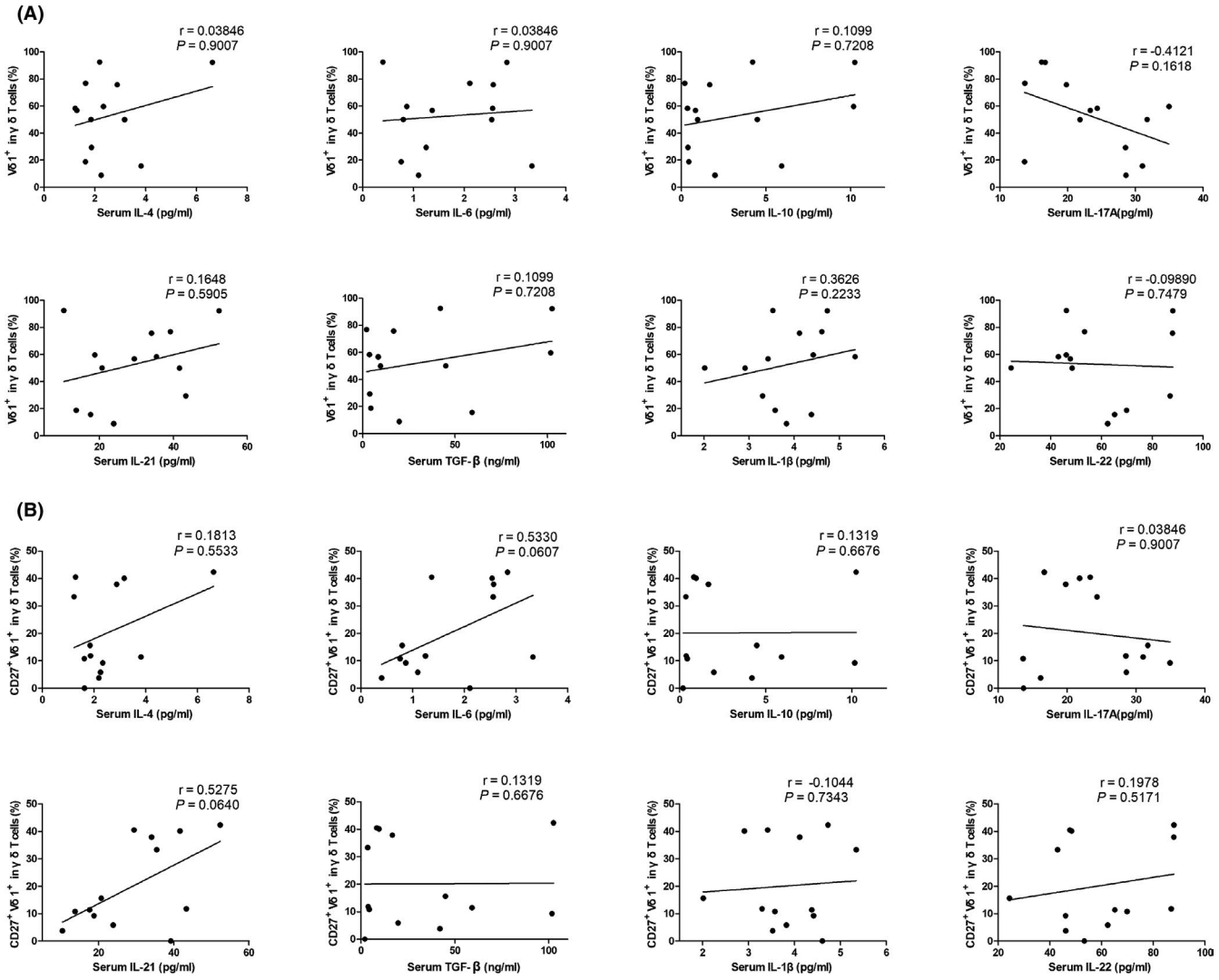

FIGURE 2 Correlation between two subtypes of γδ T cells and cytokines in patients with advanced schistosomiasis. Correlations among the proportion of the $CD27^+$ $Vδ1^+$ cells, $Vδ1^+$ cells, and the level of cytokines are shown. The cytokines included IL-4, IL-6, IL-10, IL-17A, IL-21, TGF-β, IL-1β and IL-22. None of the correlations were statistically significant

found that IL-17 concentrations were higher at the acute stage of schistosomiasis compared with that in the other stages. Our findings agreed with the reduction of IL-17 in the advanced stage of infection observed in the mouse model, which might be because the γδ T cells did not produce any IL-17 in the late stage of infection.[17]

There has only been one study of γδ T cells in patients with schistosomiasis, and that study only included patients with *S mansoni* and *S haematobium* infections, not *S japonicum* infection.[14] They observed changes in the proportion of γδ T cells among $CD3^+$ cells without structural changes of γδ T cells themselves. A previous study investigated cell functions during infections with other parasites, and showed that circulating γδ cells were impaired in human chronic infection with cystic echinococcosis.[24] γδ T cells enhanced the expression of *Plasmodium* immunogenic factors and exacerbated subsequent systemic and brain-infiltrating inflammatory αβ T cell responses.[25]

Other studies of patients infected with *S japonicum* tested cytokines in patient serum and showed that IL-21 was increased in acute and chronic infections.[26,27] However, in our study, there was no significant change in IL-21 levels in the advanced disease group. By contrast, Long et al[28] found that *S japonicum*-induced fibrotic liver tissue had higher IL-13 expression than normal liver tissue. However, IL-13 is not secreted by γδ T cells, but by traditional αβ T cells. Li et al[29] found that the mRNA levels of *TGFB1* (TGF-β1) correlated with the stage of fibrosis in patients with chronic disease; however, in the present study, there was no significant difference in serum TGF-β levels between patients with advanced schistosomiasis and the healthy controls. Perhaps, it is necessary to design another experiment, including three groups, including patients with chronic and advanced schistosomiasis, to determine the role of TGF-β. The importance of IL-10 in protecting against pathogen-induced tissue and liver injury has been demonstrated in other infectious disease

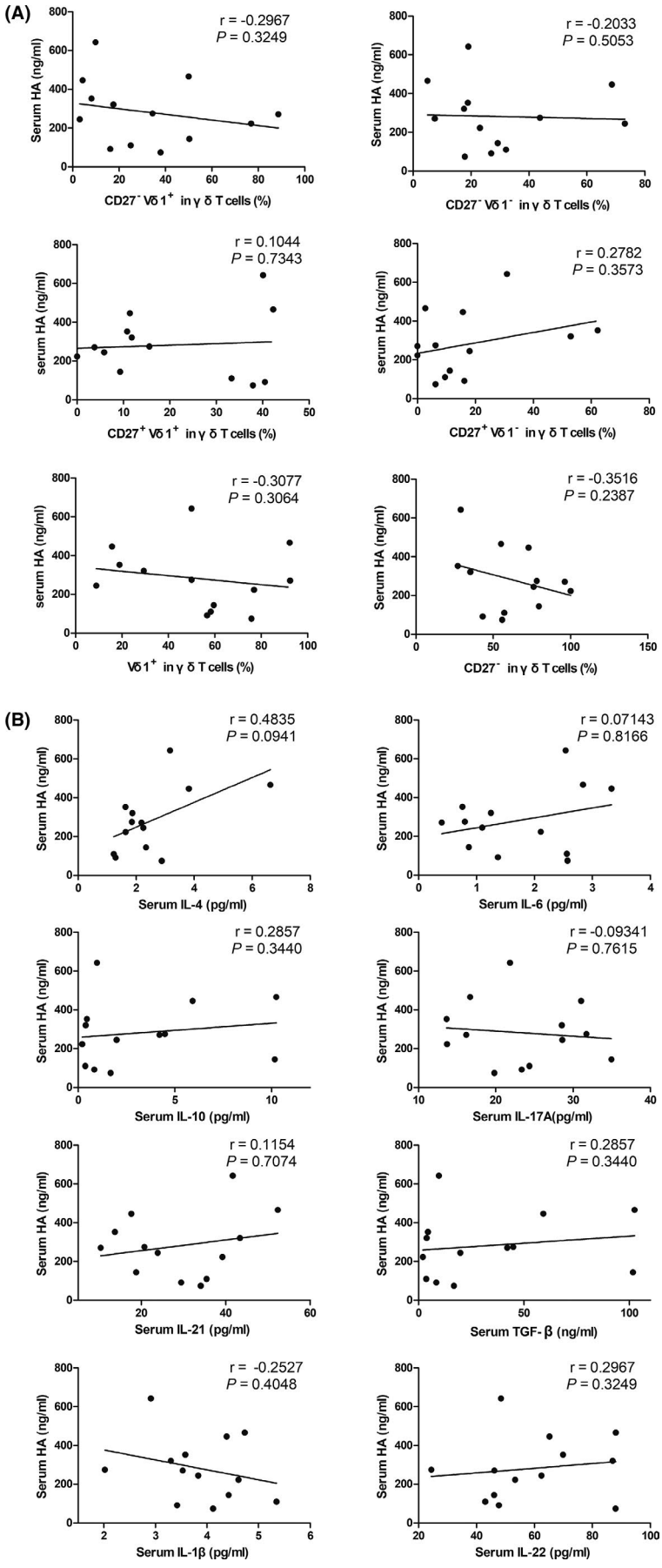

FIGURE 3 Correlations among circulating γδ T cells, cytokines, and the degree of liver fibrosis in patients with advanced schistosomiasis. (A) Correlation between the proportion of the subtypes of γδ T cells and the degree of liver fibrosis. The subtypes included the $CD27^-$ $V\delta1^+$ cells, $CD27^-$ $V\delta1^-$ cells, $CD27^+$ $V\delta1^+$ cells, $CD27^+$ $V\delta1^-$ cells, $V\delta1^+$ cells, and $CD27^-$ cells. (B) Correlation between the level of the cytokines in the serum and the degree of liver fibrosis. The cytokines included IL-4, IL-6, IL-10, IL-17A, IL-21, TGF-β, IL-1β and IL-22. None of the correlations were statistically significant

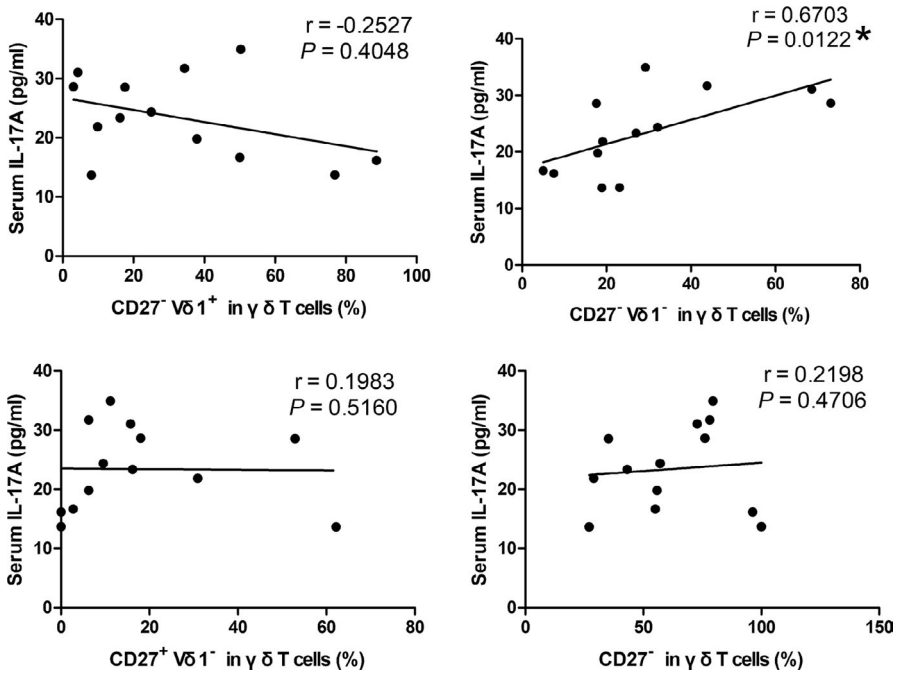

FIGURE 4 Correlations between other subtypes of γδ T cells and cytokines in patients with advanced schistosomiasis. The subtypes included the $CD27^{-}$ $V\delta1^{+}$ cells, $CD27^{-}$ $V\delta1^{-}$ cells, $CD27^{+}$ $V\delta1^{-}$ cells and $CD27^{-}$ cells. Levels of IL-17A in serum correlated significantly with the number of $CD27^{-}V\delta1^{-}$ γδ T cells. *:$P < .05$

models [30]; however, the levels of IL-10 were similar between the two groups in the present study.

In the patients with advanced schistosomiasis, the proportion of the $CD27^{-}V\delta1^{-}$ subset among total γδ T cells correlated positively with IL-17A levels in serum. The Vδ3 subtype cells are a very small proportion; therefore, if we consider the $V\delta1^{-}$ cells as Vδ2 cells, then this association suggests that the decline in IL-17A is most likely caused by a decrease in the ratio of Vδ2 cells or their activity, which implied that Vδ2 cells are the main source of IL-17A in the serum of patients with advanced schistosomiasis. Interestingly, Vδ2 cells are the main source of IL-17 in many other infections, such as by *Mycobacterium tuberculosis*[31] and human immunodeficiency virus.[32] Vδ2 T cells produce proinflammatory cytokines and chemokines, kill infected cells, secrete growth factors for epithelial cells, and present antigens to αβ T cells.[33] Besides, although we considered $V\delta1^{-}$ cells to be Vδ2 cells, the specific subtype of γδ T cells involved and their phenotypic characteristics require further in vitro and in vivo study. Further detailed classification and functional verification of the γT cells are required. In addition, whether they play a role in the pathological process of schistosomiasis, and the potential mechanism, requires further exploration.

In patients with advanced schistosomiasis, the degree of liver fibrosis increased significantly compared with that in the healthy controls. The increase of HA and PC-III in peripheral blood was related to hepatic fibrosis, which was consistent with our expectations.[34] However, in our study, we observed no direct link between the ratios of γδ T cell subsets and liver fibrosis. It is possible that γδ T cells only assist with the promotion or mitigation of fibrosis. Another possibility is that other classification methods might need to be explored to reveal the relationship between γδ T cells and liver fibrosis. Activated Vγ9+ T cells could act as anti-fibrotic mediators in systemic sclerosis, although decreased responsiveness to isopentenyl pyrophosphate might play a role in the pathological fibrosis of this disease.[19] In the late stage of schistosomiasis, there was no significant association between hepatic fibrosis and IL-17A producing γδ T cells, possibly because the body was in an immunosuppressive state, and the effector cells, including αβ cells, were losing their regulatory ability. However, although IL-17A levels were different between the two groups, there was no correlation between IL-17A and the degree of liver fibrosis. HSCs trigger robust IL-17A production by γδ T cells through the production of IL-1β and IL-23 at the early stages of liver fibrosis.[35] However, the in vivo function of cytokines that affect HSCs in vitro remains to be determined. Although we did not find any significant relationship between cytokines and fibrosis, this research still serves as a basis for future studies to discover other cytokines associated with liver fibrosis.

Several limitations need to be noted regarding the present study. First, the number of patients was low because there were no new cases of infection by *S japonicum*. in Hubei Province for 5 years, and epidemiologically, most cities in this region have been in a state of transmission blocking. Therefore, there were insufficient new cases to fully describe the progress of schistosomiasis and to determine changes in cytokines during the disease process. Secondly, it would be helpful to stain all γδ T cell subsets specifically, not only staining for one subset and assuming that the remaining cells mostly represent the other major subset (Vδ2 cells). The limited experimental conditions meant that we did not obtain intercellular staining of γδ T cell for IL-17 secretion. Further in vitro experiments should be performed to demonstrate the relationship between γδ T cells and cytokines.

It is recommended that further research be undertaken in the following areas: First, studies including acute and chronic infection groups are needed. Second, research is also required to identify more cytokines and their intrinsic link to liver fibrosis. Third, the

group of patients need to be enlarged to determine functions of γδ T cells more clearly, such as the possible correlation of IL-6 and IL-21 with $CD27^{+}V\delta1^{+}$ cells.

5 | CONCLUSION

A study of γδ T cells was carried out in patients with advanced schistosomiasis. The proportion of Vδ1+ cells and $CD27^{+}V\delta1^{+}$ cells among all γδ T cells increased. However, there was no significant correlation between the two subsets and the levels of cytokines, including IL-4, IL-6, IL-10, IL-17A, IL-21, TGF-β, IL-1β or IL-22. In addition, we found no relationship between the level of liver fibrosis and cytokines or subsets of γδ T cells. Moreover, we found that the serum level of IL-17A correlated positively with the proportion of $CD27^{-}V\delta1^{-}$ cells. The present research increased our understanding of the role of γδ T cells in the immune environment of human schistosomiasis.

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CONFLICT OF INTEREST

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript.

AUTHOR CONTRIBUTIONS

Li Zheng, Xiaorong Zhou and Jianping Cao: conceived and designed the experiments. Li Zheng: performed the experiments. Jia Yi, Lun Wan, Lixia Wang and Xiaorong Zhou: involved in sample collection. Jianping Cao and Li Zheng: contributed reagents and materials. Li Zheng, Yuan Hu and Yujuan Shen: analysed the data. Li Zheng and Jianping Cao: wrote the paper.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/pim.12871

DATA AVAILABILITY STATEMENT

All datasets generated and analysed during the current study are included in the article. Original raw data are available from the corresponding author upon reasonable request.

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