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Role of TIM-1-Positive Regulatory B Cells in the Immunopathogenesis of Hepatic Alveolar Echinococcosis: Implications for Immune Modulation and Therapeutic Targeting

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Abstract

Background: TIM-1-positive regulatory B cells (TIM-1+Bregs) are important modulators of immune responses and influence the liver's immune microenvironment. However, their role in hepatic alveolar echinococcosis (HAE) is not well understood. This study investigates the involvement of TIM-1+ Bregs in the immunopathogenesis of HAE.

Methods: Hematoxylin-Eosin (H&E) staining was used to assess liver tissue pathology and inflammatory changes. Immunohistochemistry (IHC) and quantitative real-time PCR (qRT-PCR) measured TIM-1 and IL-10 expression in liver tissues from HAE patients. Flow cytometry analyzed the frequencies, phenotypes, and functions of TIM-1+ Bregs and IL-10+-TIM-1+ Bregs in the peripheral blood of 20 HAE patients. Immunofluorescence was used to examine the colocalization of TIM-1+ B cells and TIM-4, its ligand, in liver tissues.

Results: H&E staining revealed liver architecture disruption and inflammatory infiltration in HAE lesions. IHC and qRT-PCR showed significantly higher TIM-1 and IL-10 expression in HAE tissues compared to healthy controls ($P < 0.05$ and $P < 0.01$, respectively). Flow cytometry revealed increased levels of TIM-1+ B cells, TIM-1+ regulatory B cells (Bregs), and IL-10+TIM-1+ Bregs in HAE patients' peripheral blood ($P < 0.05$). Immunofluorescence confirmed the colocalization of TIM-1+ B cells and TIM-4+ macrophages in the liver tissue, indicating a potential interaction between these cells.

Conclusions: This study identifies TIM-1 as a key immune marker in HAE and highlights the role of TIM-1+ Bregs in regu-

lating the immune response. The TIM-1/TIM-4 interaction may contribute to immune evasion by *Echinococcus multilocularis*, suggesting that targeting TIM-1+ Bregs and the TIM-1/TIM-4 pathway could offer new immunomodulatory treatment strategies for HAE.

Keywords: Alveolar echinococcosis; Immune evasion; TIM-1^{+} Bregs; IL-10

Abbreviations: AE: Alveolar Echinococcosis; hepatic AE: hepatic Alveolar Echinococcosis; HAE: hepatic alveolar echinococcosis; TIM-1: T cell Ig and mucin domain-1; TIM-4: T cell Ig and mucin domain-4; Breg: Regulatory B; IL-10: Interleukin-10; qPCR: Quantitative polymerase chain reaction; TIM-1^{+}Bregs: TIM-1-positive regulatory B cells; DAPI: 4′,6-diamidino-2-phenylindole

Introduction

Alveolar echinococcosis (AE) is a helminthic illness that causes serious health problems in humans and could even be fatal if left untreated[1]. AE mostly affects the liver， the vesicles infiltrate and expand into the surrounding liver parenchyma in a multivesicular sprouting pattern. After the sprouted vesicles detach, they can migrate to distant organs through the blood and lymphatic pathways，leading to metastases in adjacent or distant tissues/organs, which increases the severity of the disease. This phenomenon is pathologically similar to a gradually growing tumor; therefore, it is also referred to as “worm cancer”[2]. It is estimated that nearly 2 billion people have been infected by helminths with approximately 200 million cases of echinococcosis have been recorded worldwide, and 0.3% of these are reportedly caused by AE[3]. Studies have suggested that the process of being infected with *Echinococcus multilocularis* involves a complex immune escape reaction. This immune escape by *E. multilocularis* within the host is characterized by a steady reduction in the host’s immune state, which makes the infection appear chronic. On the other hand, the regulatory mechanism of immune escape requires further investigation[4]. Identifying the main immune regulatory cells involved in this immune escape mechanism would assist in deciphering the signaling pathway underlying this immune escape in hepatic hydatid disease.

In AE, the immune cell microenvironment plays a critical role in regulating immunological responses[5]. Currently,there is no immunotherapy for AE,and existing treatments primarily focused on T-lymphocytes (T cells), dendritic cells (DC), T regulatory, and macrophages[6]. Gradually, B-lymphocytes (B cells) are also being recognized as important participants in immunity and treatment processes. B cells are well recognized for their ability to produce antibodies that actively regulate immunological and inflammatory responses,and these antibodies also stimulate T cell activation and proliferation in the presence of antigens[7]. However, the existing knowledge regarding B cells in relation to echinococcosis is scarce.

B cells are well recognized for their ability to produce antibodies that actively regulate immunological and inflammatory responses. These antibodies also stimulate T cell activation and proliferation in the presence of antigens[8]. However, investigations conducted over the last decade have demonstrated that different subsets of immunoregulatory B cells exist in mice and humans. Regulatory B (Breg) cells, a subset of suppressor B cells, play a crucial role in maintaining immunological tolerance[9]. They reduce pathological autoimmune and inflammatory responses, as well as modulate immune surveillance, by releasing anti-inflammatory mediators, such as interleukin-10 (IL-10)[10]. IL-10 is a powerful anti-inflammatory cytokine that helps maintain the balance of immune responses and thereby prevents the development of chronic inflammatory diseases[11]. Increased IL-10 expression skews the Th2 response, facilitating parasite multiplication by modulating the balance between T helper cell type 1 (Th1) and T helper cell type 2 (Th2)[12]. This balance is a significant research focus in understanding immunological escape mechanisms in helminthic diseases.

T cell Ig and mucin domain (TIM)-1 is a transmembrane glycoprotein and one of three members of the human TIM-1 family

of genes that govern immunological responses[13]. Initially, TIM-1 was reported to play a key role in the activation of $CD4^+$T lymphocytes and DCs. TIM-1 was also reported to regulate cellular activity, demonstrating a significant effect on immune cell function[14]. However, recent studies have shown that TIM-1 is highly expressed in B cells and serves as a Breg marker[15]. TIM-1^+Breg cells appear to include the largest proportion (over 70%) of IL-10-producing B cells, with 8~20 times higher expression of IL-10 compared to other B cell subsets[16]. Studies have explored the regulatory functions of TIM-1^+Breg cells and their relationship with systemic autoimmune diseases or immune evasion[17]. However, the biological role of TIM-1+Breg cells in the microenvironment of hepatic AE (hepatic alveolar echinococcosis; HAE) remains to be elucidated. TIM-4 is one of the natural ligands of TIM-1 and is expressed by macrophages[18]. Increasing evidence suggests that TIM-4+cells invade human tumors in close vicinity to TIM-1+B cells, indicating that interactions between TIM-1 and TIM-4 may encourage TIM-1+Breg to release IL-10[19]. Moreover, the interaction between TIM-1 and TIM-4 has been demonstrated to lower the expression of TIM-1, which then reduces the production of the immunosuppressant cytokine IL-10 in TIM-1+Breg cells[20]. However, whether TIM-4+ macrophages and B cells are colocalized within the hepatic AE microenvironment. Thus, we hypothesize that TIM-1+Breg cells enhance immunological tolerance through IL-10-dependent pathways. In this context, the present study aimed to investigate the expression of TIM-1 in the liver tissues and serum of patients with hepatic AE and also determine whether TIM-1 and TIM-4 are colocalized in the hepatic AE microenvironment.

Materials and Methods

Study Subjects

A total of 33 patients diagnosed with hepatic AE at the Department of Hepatobiliary Inclusion Surgery, First Affiliated Hospital, Xinjiang Medical University, between January 2019 and October 2021, were included in the present study. The patient population comprised 14 males and 19 females, with an average age of 39.1 years (age range, 15–59 years). The inclusion criteria were as follows: diagnosis of AE based on the 2019 Expert Consensus on the Diagnosis and Treatment of Echinococcosis; the diagnosis was confirmed through medical history, clinical symptoms, laboratory and imaging examination results, and the gold standard diagnostic method for echinococcosis. Patients with chronic infectious diseases (due to bacteria, viruses, etc.), malignant tumors, rheumatic immunological illnesses, cystic echinococcosis, or other parasitic disease, as well as those who consumed non-steroidal anti-inflammatory drugs, hormone drugs, psychotropic drugs, etc., were excluded from the study. In addition, 33 aged-matched healthy controls were selected from blood donors at the hospital, comprising 14 males and 19 females, with an average age of 43.7 years (age range 13–71 years). No evident abnormalities were detected in the blood tests, electrocardiogram, and B-ultrasound results. All included individuals provided informed written consent prior to the study, and for minors (under the age of 18) written informed consent was provided by their respective parents or legal guardians.

Tissue and Blood Collection and Biochemical Analysis

In patients with hepatic AE, liver tissues were surgically obtained from within 2 cm of the lesion for the experimental group, whereas the liver tissues were collected 2 cm outsides the lesion for the control group (I refer to the article？)[21]. Parts of the liver tissues were fixed in 10% formaldehyde, followed by paraffin-embedding and sectioning into 3 μm slices, which were subsequently subjected to H&E staining and immunohistochemistry analysis. The remaining portions of the liver samples were stored at –80 °C until used for qRT-PCR detection. Blood samples were obtained from the hepatic patients as well as from age--matched healthy controls and subjected to biochemical parameters evaluation using established techniques. The serum levels of total bilirubin (TBil), aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transpeptidase (GGT), and alkaline phosphatase (ALP) were determined using an automatic blood biochemical analyzer (Beckman Counter LX20, USA), are presented in Table 1.

Table 1: Clinical characteristics of the study subjects (Mean ±SD)

	AE (*n* = 33)	HC (n = 33)
age(Average)	39.1	43.7
Sex ratio		
Female: Male	14:19	14:19
nation		
Ethnic Han	8	22
Uyghur	0	10
Tibetan	13	0
Other	12	1
Eosinophilic granulocyte (%)	3.15 ±3.11**	2.05 ±1.91
Liver function		
TBil(μmol/L)	39.47 ±46.43**	14.89 ±7.34
AST(U/L)	437.02 ±1070.22***	25.70 ±20.10
ALT(U/L)	410.47 ±922.45***	23.57 ±18.55
GGT(U/L)	139.68 ±141.58***	30.62 ±27.53
ALP(U/L)	360.01 ±730.94***	70.11 ±21.24

***P <0.001, **P<0.01.

H&E Staining and Immunohistochemistry

The liver tissue sections, with a thickness of 3 μm, were stained with hematoxylin-eosin (H&E) and immunohistochemically (IHC) using the respective standard procedures. The stained samples were then examined and photographed under a microscope (OLYMPUS BX43, Japan). Based on the chronic inflammation scores, a pathology score was developed in a blinded manner to assess the severity of liver inflammatory lesions in patients with hepatic AE using a microscope (OLYMPUS BX43). For the immunohistochemistry analysis, the sections were incubated overnight at 4 °C with primary antibodies (anti-IL-10 antibody, 1:100 dilution; anti-TIM-1 antibody, 1:200 dilution; Abcam). Afterward, the sections were incubated with the secondary antibody (biotinylated goat anti-rabbit lgG; Mavision™, Maxim China) for 30 min. Subsequently, the sections were counterstained with hematoxylin, covered with coverslips, and examined under a microscope (OLYMPUS BX43, Japan), followed by capturing suitable images.

RNA Extraction and Real-Time PCR

RNA was extracted using the TRIzol™ isolation kit (Takara Bio, Dalian, China) according to the manufacturer's instructions, followed by cDNA synthesis using Primer Script RT kit (Takara Bio, Dalian, China). The primers for GAPDH, IL-10, and TIM-1 were synthesized by Sangon Biotech (Shanghai, China), and the primer sequences are presented in Table 2. Real-time PCR was then performed on the ABI Prism 7500 Sequence Detection System (Applied BioRad, Hercules, CA, USA) using the following reaction conditions: one cycle at 95 °C for 5 s, followed by cycles at 64 °C for 30 min. All reactions were conducted in triplicate for each sample. The $2^{-\Delta\Delta ct}$ method was adopted to calculate the relative concentration of each target, and for normalization, GAPDH level was used as the internal reference.

Table 2: Primer sequences

Gene (human)	Primer sequence
IL-10TIM-1	F: GGGAGAACCTGAAGACCCTCA R: TGCTCTTGTTTTCACAGGGAAGF: TTGAGAAGAGTTACGAGTTG R: GGACATTGTTAGCATAGAGG
GAPDH	F: CATCCACTGGTGCTGCCAAGGCTGT
	R: ACA ACCTGGTCCTCAGTGTAGCCCA

Immunofluorescence

In the immunofluorescence analysis, paraffin-embedded human hepatic AE sections were incubated overnight with the primary antibody (anti-CD20 antibody – 1:200; anti-TIM-4 antibody – 1:400; Abcam) at 4 °C, followed by which the samples were left undisturbed for 1 hour at room temperature and then incubated with the corresponding secondary antibody, which was added dropwise, at room temperature for 1 hour. Afterward, the DAPI(4′,6-diamidino-2-phenylindole) fluorescent dye was developed, followed by a wash with PBS. The DAPI-positive cells were examined and photographed under a fluorescence microscope.

Flow Cytometry

Venous blood samples were collected from both AE patients and healthy controls, and single nucleated cells (PBMCs) were isolated using gradient centrifugation. After washing and counting, PBMCs were cultured in 6-well plates with a complete medium at a density of 1.5–2.0 × 10^6 cells per well. Lipopolysaccharide (LPS) was added to stimulate the cells for 4 hours. The culture supernatant was discarded, and the cell pellet was stored at −20 °C with DMSO. The pellet was then resuspended in PBS and stained with surface antibodies (FITC-anti-CD19, Percp-5–5-anti-CD24, PE/Cy7-anti-CD38, APC-anti-TIM-1) for 25 minutes. After washing, membrane breaking was induced, followed by intracellular staining with PE-anti-IL-10. The samples were analyzed using a BD flow cytometer II, with data processed by FLOWJO software. Each sample contained over 30,000 cells.

Statistical Analysis

All data were analyzed statistically using SPSS 21.0 (IBM, Chicago, IL, USA). Graphs were plotted using GraphPad Prism 6.0 software. The measurement data were expressed as the mean ±SEM. An independent samples t-test was used to compare the means between two groups. The Wilcoxon test was used as a non-parametric alternative. Pearson's correlation was used to analysis the correlation. Statistical significance was indicated by a p-value of <0.05.

Results

Biochemical Parameters of the Patients with Hepatic AE

Liver function parameters in patients with hepatic AE were significantly elevated compared to the healthy control group. Total bilirubin levels, along with liver enzymes such as AST, ALT, and GGT, were markedly higher in AE patients. Alkaline phosphatase levels were also notably increased in the AE group compared to healthy controls. These findings suggest significant liver dysfunction in AE patients, as reflected by the elevated levels of liver enzymes and bilirubin, indicating impaired hepatocyte function and a highly imbalanced liver microenvironment (Table 1).

Pathological Changes Were Observed in the Liver Lesions of Patients with Hepatic AE

The hepatic AE samples stained with H&E exhibited abnormal morphology. The healthy controls displayed a normal hepatic

lobule structure, with the central vein visible within the tissue. Hepatocytes were organized radially around the central vein, the hepatic sinus anatomy was clear, and no pathological change were observed. In contrast, patients with hepatic AE showed a damaged lobular structure, with several inflammatory lesions present, surrounded by numerous infiltrating inflammatory cells. Significant congestion was visible in the central vein, and the hepatic sinus was evidently dilated (Figure 1). Therefore, inflammation scores were computed for both the hepatic AE patients and healthy controls, and the results (Table 3) revealed that patients with hepatic AE had higher inflammatory scores compared to the healthy controls.

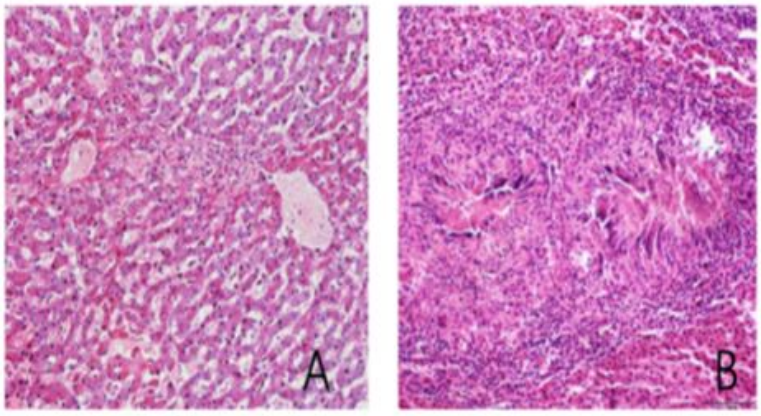

Figure 1: Results of H&E staining of the liver lesion in patients with hepatic AE (magnification, 200×):(A) control group; (B) HAE group

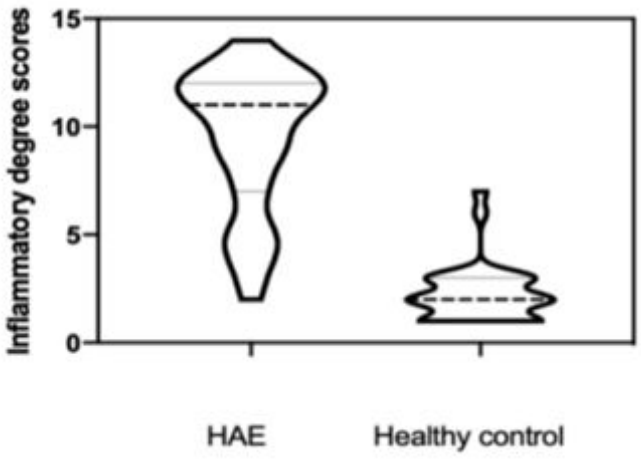

Table 3: The pathological scores for the inflammatory changes in the liver tissue of patients with AE

Results of the Immunohistochemical Staining of the Liver Tissue

The IHC staining for IL-10 revealed a brownish mass around the liver lision tissue, with IL-10-positivity detected mainly in the cytoplasmic region of the cells, while the controls exhibited reduced IL-10 positivity (Figure 2). TIM-1, a Breg surface marker, resulted in dark-yellow staining in the cytoplasm in the IHC analysis, while the control group exhibited dramatically decreased TIM-1-positive staining. Moreover, TIM-1 and the anti-inflammatory cytokine IL-10 were differentially expressed between the two groups ($P < 0.01$, Table 4).

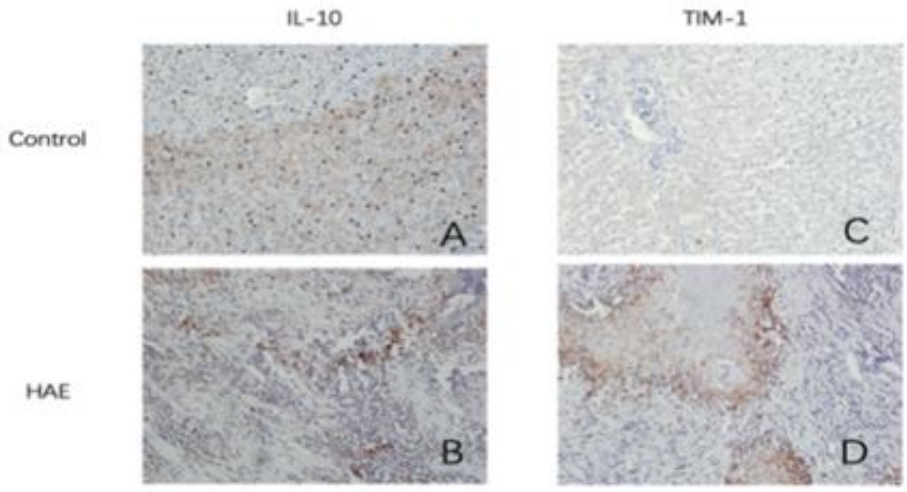

Figure 2: Results of immunohistochemical staining of liver tissues from patients with hepatic AE (magnification, 200×).

(A) Weak IL-10 positivity or negativity was detected in the cytoplasm of the control group.

(B) Cytoplasmic expression of IL-10 was strongly positive in the hepatic AE group.

(C) TIM-1 was weakly positive or negative in the cytoplasm in the control group.

(D) Cytoplasmic expression of TIM-1 was considerably positive in the hepatic AE group.

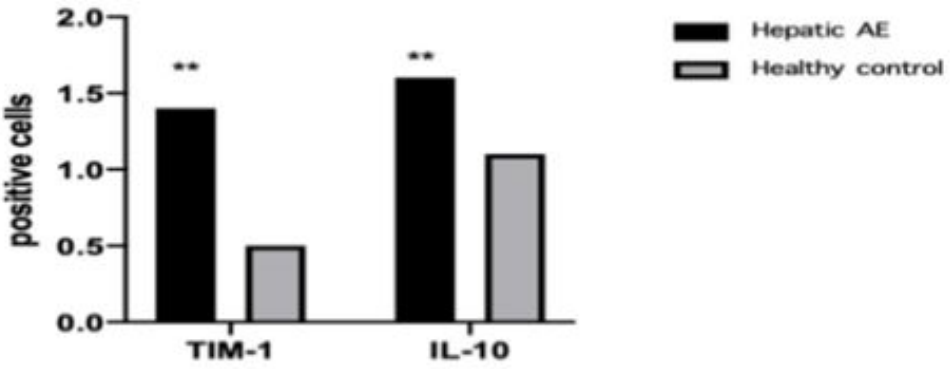

Table 4: The immunohistochemical staining results depicting stain-positive cells in the liver tissues of patients with hepatic AE (magnification, 400×). TIM-1 and the anti-inflammatory cytokine IL-10 were differentially expressed between the two groups. (**P < 0.01, compared to the control group).

TIM-1 mRNA levels and IL-10 mRNA levels are associated with the development of hepatic AE

The qRT-PCR analysis results revealed that the expressions of Tim-1 and IL-10 in the hepatic AE patient group were significantly different from those in the control group ($P < 0.05$, Table 5). TIM -1 was positively correlated with ALP, GGT, and Tim-1, suggesting its potential as a marker for hepatic AE infection (Figure 3).

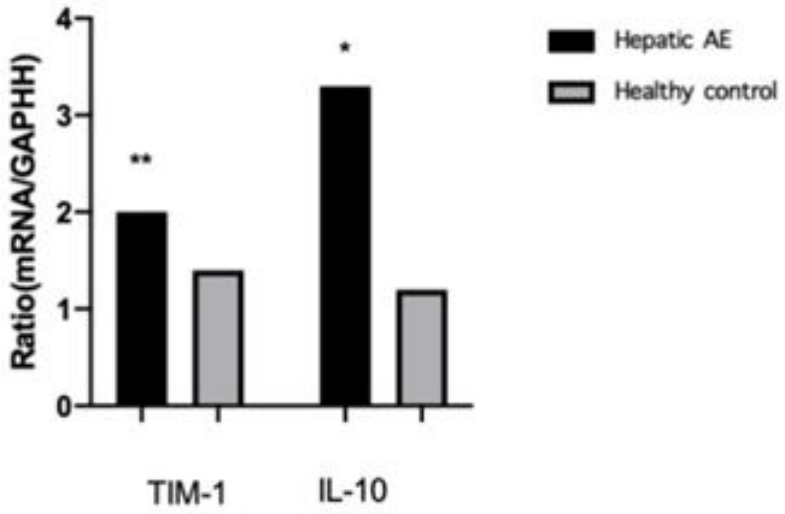

Table 5: The gene expression levels of TIM-1 and IL-10 in the hepatic AE and healthy control groups (*P < 0.05 and *P < 0.01).

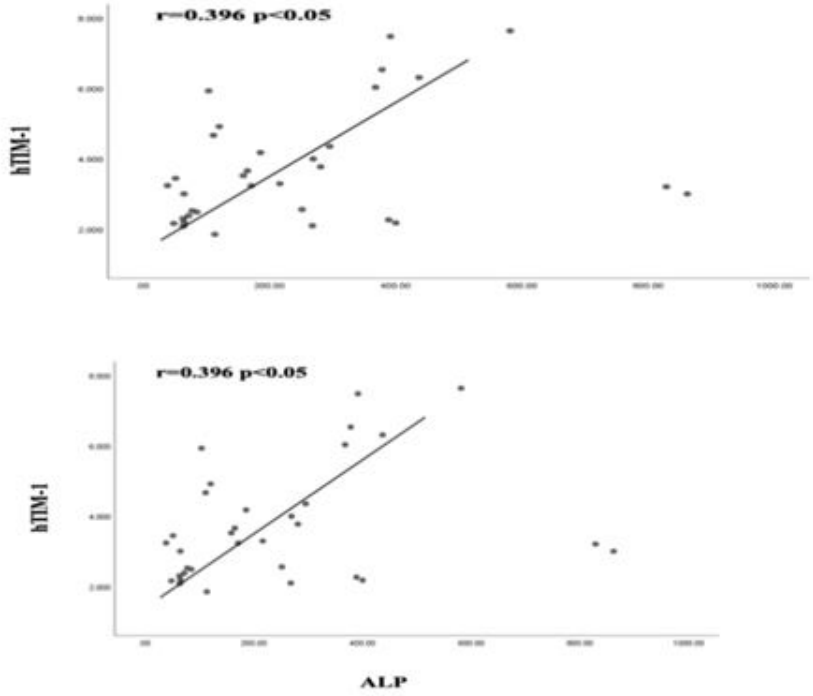

Figure 3: In Correlation Analysis, R refers to the Correlation Coefficient. It is a statistical measure that indicates the strength and direction of the linear relationship between two variables. The closer R is to 1, the stronger the positive relationship between the two variables. In the AE patient group, Tim-1 was positively correlated with ALP and GGT (*P < 0.05 and *P < 0.01).

Proportion of total CD19+CD24hiCD38hiTIM-1-IL-10 B cells (TIM-1+IL-10+Breg cells)

First, we used flow cytometry to identify $CD19^+$ B cells and further selected a subpopulation of cells with high expression of CD24 (CD24hi) and CD38 (CD38hi). These cells were defined as $CD19^+$CD24hiCD38hi B cells, also known as regulatory B cells (Breg cells). The gating strategy is illustrated in Figure 4 through representative flow cytometry dot plots. After confirming the identification of $CD19^+$ B cells and the gating of Breg cells, we analyzed two specific Breg cell subpopulations: Breg cells expressing TIM-1 (TIM-1^+ Breg) and Breg cells secreting IL-10 (IL-10^+ Breg). We measured the proportions of these two subpop-

ulations in the peripheral blood of 20 healthy controls and 23 AE patients. The results showed that the proportions of TIM-1$^+$ Breg and IL-10$^+$ Breg cells in the peripheral blood of AE patients were significantly higher than those in healthy controls ($P < 0.05$; detailed data are shown in Figure 5 and Table 6). This suggests that these Breg cell subpopulations may be closely associated with the pathological state of AE patients. To more comprehensively assess the changes in these Breg cell subpopulations, we combined the proportions of TIM-1$^+$ Breg and IL-10$^+$ Breg cells to calculate the proportion of TIM-1$^+$IL-10$^+$ Breg cells. The results indicated that this combined index was also significantly higher in AE patients than in healthy controls ($P < 0.05$; detailed data are shown in Figure 6 and Table 7). This further confirms the characteristic changes of TIM-1$^+$ and IL-10$^+$ Breg cells in AE patients.

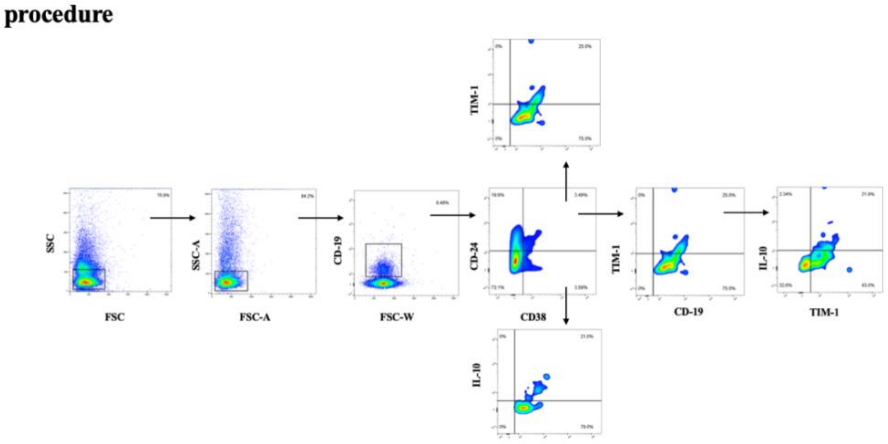

Figure 4: The flow cytometry dots are displayed layer by layer

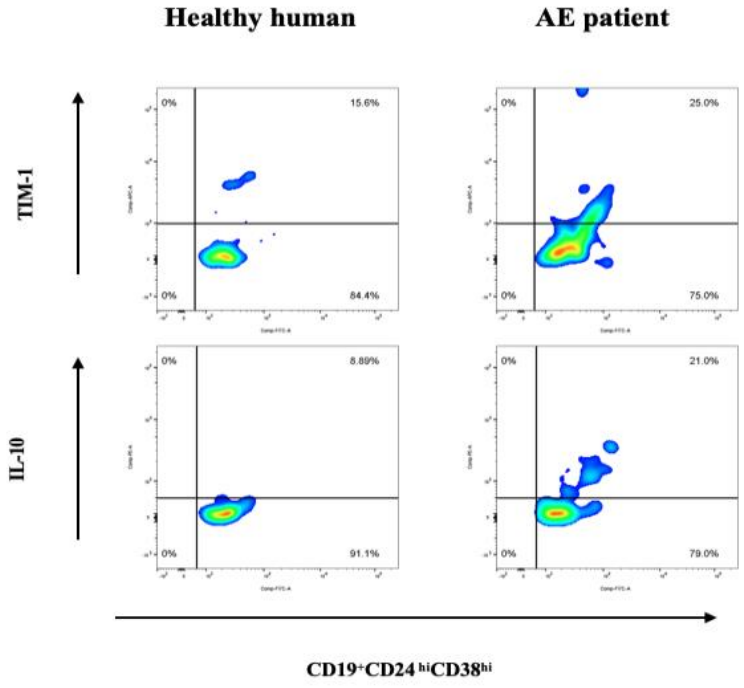

Figure 5: Cells were surface stained using monoclonal antibodies for CD19, CD24, CD38, and TIM-1 (mAbs), followed by intracellular staining for IL-10.

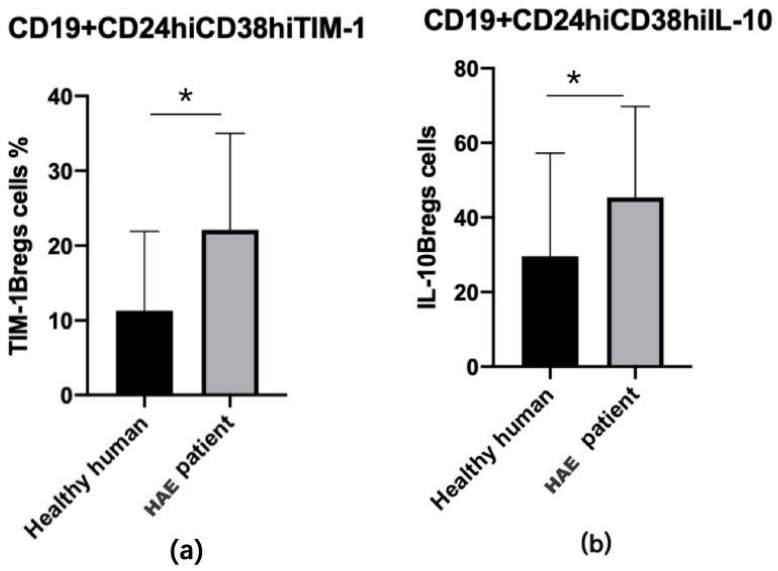

Table 6: Histogram depicting the percentages of $CD19^{+}CD24^{hi}CD38^{hi}$TIM-1 and $CD19^{+}CD24^{hi}CD38^{hi}$IL-10 cells, which were significantly higher in the AE group than those in the control group (hepatic AE = 23, HC = 20, *P < 0.05).

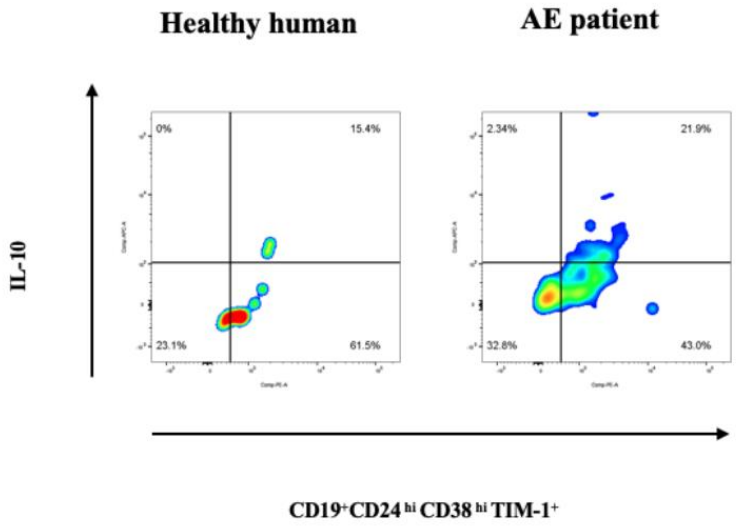

Figure 6: Representative intracellular IL-10 staining in the unstimulated $CD19^{+}CD24^{hi}CD38^{hi}$TIM-1B cells in the peripheral blood of the hepatic AE patients and healthy controls.

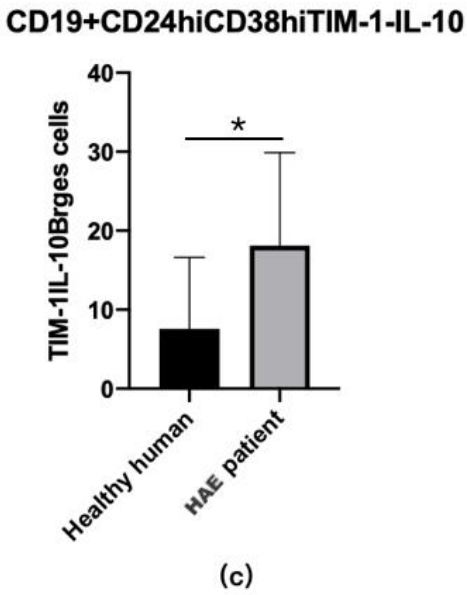

Table 7: The statistical graph illustrating the frequency of IL-10+ cells in stimulated CD19+CD24hiCD38hiTIM-1B cells of the hepatic AE patients and healthy controls (*P < 0.05).

Immunofluorescence Revealed the Co-Localization of TIM-1 and Tim-4 in Hepatic Alveolar Echinococcosis

In the present study, the results of confocal microscopy revealed that Tim-4$^+$ macrophages and B cells were located in the same region (Figure 7), which indicated that the TIM-1/Tim-4 signal could be involved in promoting the production of IL-10 in Tim-1$^+$ B cells.

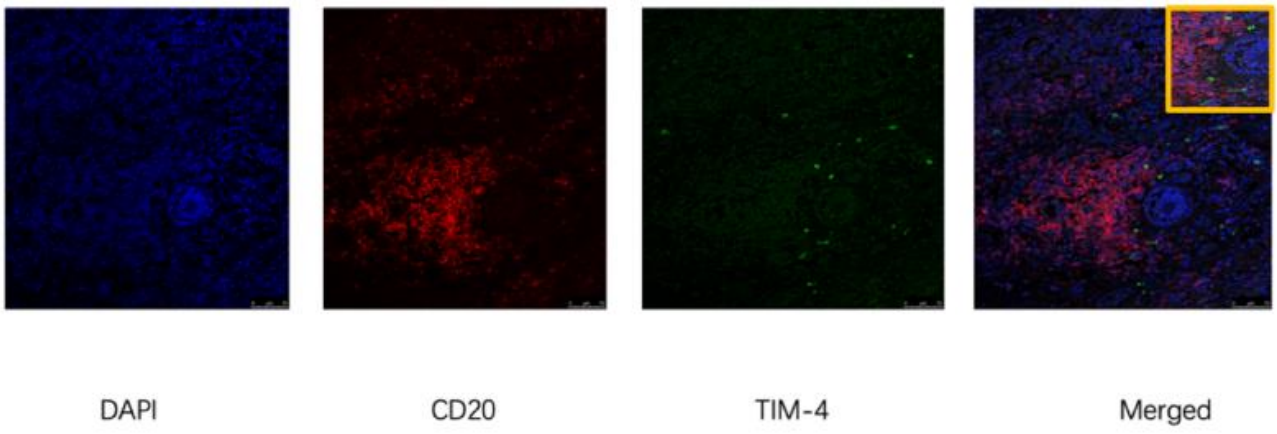

Figure 7: Representative immunofluorescence images of CD20 (red), TIM-4 (green), and nuclear staining with DAPI (blue) for the HAE tissue (The yellow box shows the phenomenon of co-localization of TIM-1 and TIM-4.).

Discussion

AE is a severe disease transmitted between animals and humans, characterized by ongoing and progressive liver damage due to

the persistent growth of the larval form (metacestode) of *E. multilocularis* [1]. While less common than cystic echinococcosis (CE), AE has garnered more attention in the medical field recently. Similar to CE, AE often does not show symptoms in its early stages, and the lesions can be asymptomatic for 10 to 15 years. Chronic inflammation in AE may develop into fibrosis, which could lead to central calcification[22]. If untreated, AE is highly fatal, with an estimated 94% of patients dying within 10 to 20 years after diagnosis[23].

The present study provides novel insights into the immunopathogenesis of hepatic alveolar echinococcosis (HAE) by focusing on the role of TIM-1-positive regulatory B cells (TIM-1+ Bregs) and their association with IL-10 expression in the liver microenvironment. Our findings reveal a significant increase in TIM-1 and IL-10 expression in HAE patients, suggesting a potential immune regulatory mechanism involving these elements. The results showed that compared to the healthy control group, HAE patients exhibited evident pathological changes in their liver tissues (Figure 1). In addition, the inflammation scores for cholestasis, calcification, vesicles (number), necrotic liquescence cavity (number), and inflammatory cell infiltration were compared between the HAE patients and the healthy controls. Our results indicate that HAE is associated with a disrupted hepatic microenvironment, as evidenced by the biochemical parameters and the pathological changes observed in liver tissues. Increase levels of AST, ALT, and GGT in HAE patients suggest hepatocyte dysfunction and a disrupted liver microenvironment, which is further supported by the H&E staining results showing inflammatory infiltration and structural damage in the liver tissues of HAE patients. Next, basic information regarding the patients, the percentage of eosinophil granulocytes, and liver function measures was collected (Table 1). The liver function in hepatic AE patients was severely impaired.

Clinical observations and animal experiments have indicated that fully effective anti-parasitic chemotherapy for AE patients is lacking. Therefore, immunotherapy aimed at modulating the host immune response could serve as an attractive alternative or adjuvant therapy.

In this context, understanding the cellular and molecular basis of hepatic AE -related immunomodulation is a prerequisite for developing effective immunotherapy-based treatment strategies against the disease caused by this deadly parasite.

Echinococcus multilocularis is a parasite capable of surviving for extended periods inside the host, where it uses the host's nutrients for its proliferation and transfer and exhibits multiple complex immune evasion mechanisms. B cells, which are an important component of humoral immunity, play a role in immunoglobulin (Ig) production, antigen presentation, and secretion of pro-inflammatory cytokines. Regulatory B (Breg) cells, a subset of these B cells, exhibit immunosuppressive effects through the production of regulatory cytokines, such as IL-10, IL-35, and transforming growth factor-β (TGF-β)[24]. Studies have confirmed that the immune system in the body could be altered in several ways, such as the secretion of the immunosuppressive cytokine IL-10. It is suggested that TIM-1+B cells perform immunosuppressive functions by secreting IL-10[25]. Flow cytometry analyses revealed an increased proportion of TIM-1+ Bregs and IL-10+ Bregs in the peripheral blood of HAE patients. This suggests that TIM-1+ Bregs may play a role in the immune evasion strategy of *E. multilocularis* by promoting a Th2-type immune response, which is characterized by increased IL-10 production. The immunofluorescence analysis, which confirmed the colocalization of TIM-1 (B cells) and TIM-4 in the liver of HAE patients, provides further evidence for the interaction between TIM-1 and its ligand TIM-4, potentially influencing the function of TIM-1+ Bregs (Figure 7).

Tim-1+Breg cells appear to include a high proportion of IL-10-producing B cells (70%), and the concentration of IL-10 expression in these cells is 8–20 times higher than that in all other B cell subpopulations[26]. It is reported that in autoimmune diseases, $CD19^{+}Tim1^{+}$ B cells downregulate the autoimmune response by secreting the anti-inflammatory cytokine interleukin-10[27]. However, none of the existing studies have elucidated the biological function of $Tim\text{-}1^{+}$ Breg in HAE. Whether $Tim1^{+}$ $Il\text{-}10^{+}$ Breg cells are involved in the regulation of immune tolerance in AE patients remains unclear to date. The immunohistochemical staining and qRT-PCR analyses confirmed the elevated expression of TIM-1 and IL-10 in the liver tissues of

HAE patients. These findings are consistent with the role of TIM-1+ Bregs in modulating immune responses, particularly through the release of IL-10, an anti-inflammatory cytokine. The positive correlation between TIM-1 and liver function indices ALP and GGT further supports the involvement of TIM-1 in the pathogenesis of HAE(figure 3). The results suggest that in AE infection, Tim-1 protein were activated in the liver tissue of the patients, and IL-10 was released in large quantities by the immune cells during the immune response.

The growth, multiplication, and survival of *E. multilocularis* in vivo depend on effective immune escape mechanisms. Immune escape is a complex process, and one of its main mechanisms involve the induction of an imbalanced Th1/Th2 immune response in the host, initiation of apoptosis, dysregulation in $CD8^+$ T cells, and suppression of the host's anti-microbial immunogenically response. Most reports in the literature suggest that *Echinococcus* may evade clearance by the host immune system by disrupting the Th1/Th2 balance, suppressing the Th1 response, and inducing a Th2 immune response[28]. IL-10^+ B cells have been detected in the spleen of mice susceptible to atherosclerosis, and these cells can inhibit Th1 responses *in vitro* in an IL-10--mediated manner[29]. Research shows that TIM-1/TIM-4 interaction enhances IL-10 release from TIM-1^+ Bregs, contributing to the Th2-skewed immune response in HAE. This Th2 dominance can suppress Th1 immunity, which is vital for fighting intracellular parasites like *Echinococcus multilocularis*. The TIM-1/TIM-4 axis, therefore, could be a novel therapeutic target for HAE. Intervening in this axis may help rebalance Th1/Th2 responses and boost host defenses. This finding offers new treatment strategies for HAE and insights into parasite-host immune interactions(table 4、5； figure 7).

TIM-4 is the natural ligand of TIM-1 and is present in macrophages[30]. Immunofluorescence experiments in the present study revealed that Tim-4 was mostly expressed on macrophages throughout the experiment (Figure 8). Tim-1^+Breg cells may generate IL-10 through the TIM-1/Tim-4 signaling pathway. These findings suggest that Tim-1 can attract its ligand, TIM-4, on macrophages, which are consequently induced to produce greater amounts of IL-10, thereby contributing to the maintenance of the Th2-dominated immune response linked to AE development. Accordingly, it was hypothesized that preventing the binding between Tim-1 and Tim-4 could prevent the release of IL-10 via the Tim-1^+ Breg pathway. Future studies could, therefore, test this hypothesis using animal gene knockout experiments and cell-based studies.

Conclusions

The present study demonstrates that TIM-1 is expressed in patients with HAE and is significantly correlated with liver function markers, particularly ALP and GGT ($P < 0.05$), suggesting its potential as a biomarker for monitoring disease progression. These findings underscore the important role of TIM-1 in the immunopathogenesis of HAE. Future research should focus on elucidating the precise mechanisms by which TIM-1+ regulatory B cells (Bregs) contribute to immune dysregulation in HAE. Specifically, the signaling pathways involved in the interaction between TIM-1 and its ligand TIM-4 need to be further investigated, as this interaction likely mediates the release of IL-10 and skews the immune response towards a Th2-dominant phenotype. Moreover, targeting TIM-1+ Bregs could offer a promising therapeutic strategy. Preclinical models should explore this avenue to develop novel immunomodulatory treatments aimed at restoring immune balance and improving outcomes for HAE patients.

Declarations

Ethical Approval Statement

Declaration of Participation of Minors

Prior written and informed consent was obtained from patients involved in the study. The minor participants (< 18 years old)

had their informed consents signed by their parents/legal guardians.

Consent for Publication

Not applicable

Availability of Data and Materials

The data supporting the conclusions of this manuscript are included within the manuscript and are available from the corresponding author on reasonable request; RNA primer sequence can be found in the attachment.

Competing Interests

The authors declare that they have no competing interests

Declaration about Methods

We confirmed that all methods were performed in accordance with the relevant guidelines and regulations

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Authors' contributions

Aibibula conceived the study, participated in the study design, and drafted the manuscript. Qi, Li and Tian performed experiments, analyzed data, and reviewed the manuscript. Zhao supervised the experiments; Fan, Zhang and Ma helped to modify the manuscript; Shen and Xiumin Ma conceived of the study and modified the manuscript. All authors read and approved the final manuscript.

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