TIM-1+ B Cells are more Efficient IL-10 Producers in Liver Fibrosis Caused by Alveolar Echinococcosis

Bin Li
Tumor Hospital Affiliated to Xinjiang Medical University, First Affiliated Hospital of Xinjiang Medical University, Medicine of Xinjiang Medical University

Xinwei Qi
First Affiliated Hospital of Xinjiang Medical University

Tao Jiang
Xinjiang Medical University

Yumei Liu
First Affiliated Hospital of Xinjiang Medical University

Jiaoyu Shan
College of Basic Medicine of Xinjiang Medical University

Xuanlin Cai
College of Basic Medicine of Xinjiang Medical University

Jie Lv
Tumor Hospital Affiliated to Xinjiang Medical University, First Affiliated Hospital of Xinjiang Medical University, Medicine of Xinjiang Medical University

Maidinaimu Aibibula
Tumor Hospital Affiliated to Xinjiang Medical University, First Affiliated Hospital of Xinjiang Medical University, Medicine of Xinjiang Medical University

Jun Cao
First Affiliated Hospital of Xinjiang Medical University

Xiumin Ma (maxiumin1210@sohu.com)
Tumor Hospital Affiliated to Xinjiang Medical University, First Affiliated Hospital of Xinjiang Medical University, Medicine of Xinjiang Medical University

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Research Article

Keywords: Echinococcus multilocularis infection, TIM-1+B cells, IL-10

Posted Date: July 9th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-646608/v1

Abstract

Background: The cestode *Echinococcus multilocularis* (*E. multilocularis*) infection, a serious health problem worldwide, causes alveolar echinococcosis (AE), a tumor-like disease predominantly located in the liver and able to spread to any organs. Until now, there have been few studies that explain how TIM-1^+B cells contribute to the immune tolerance in *E. multilocularis* infection.

Methods: Hematoxylin-eosin (H&E) and Masson staining were used to assess the pathological inflammatory changes and collagen deposition respectively in the liver of *E. multilocularis* infected mice. Desmin, TIM-1 and IL-10 were detected by immunohistochemistry (IHC). qRT-PCR and ELISA were used to detect the IL-10 of liver and serum, respectively. Flow cytometry was used to assess the level and function of $CD19^+$TIM-1^+ cells and $CD19^+CD5^+CD1d^{hi}$ cells in spleen. Immunofluorescence (IF) were used to detect the co-localization of immune cells in the liver.

Results: At 180 days after infection, inflammatory cells and fibrosis could be observed in the liver of mice in the infection group, a large number of activated hepatic stellate cells (HSC) and TIM-1^+ cells appeared around the lesion and the expression of IL-10 was also significantly higher than that in control group. The level and function of $CD19^+$TIM-1^+ cells and $CD19^+CD5^+CD1d^{hi}$ cells in the spleen were up-regulated in the infection group, but $CD19^+$TIM-1^+ cells produced IL-10 more efficiently and could be recruited to the lesion.

Conclusions: In the late stage of alveolar echinococcosis, $CD19^+$TIM-1^+ cells can be recruited to the lesion and may be more efficient IL-10 producers to participate in the formation of immune tolerance.

Background

Alveolar echinococcosis is a rare zoonotic disease caused by *Echinococcus multilocularis*. Humans are generally not directly involved in the transmission of AE. In 98% of cases, the infection mainly exists in the liver, showing like tumor disease. It is estimated that there are over 18,000 cases of alveolar echinococcosis in the world each year, of which 91% occur in China [1]. Patients with this disease have non-specific symptoms such as upper abdominal discomfort, loss of appetite and jaundice in the early stage. The various complications in the late stage are mostly caused by the compression or damage of organs by one or more vesicles, even the volume of the vesicle is not very large [2]. There are thin or thick layers of collagen fibers around these vesicles. In the early stages of infection, these fiber layers will prevent the continued enlargement of the lesions. But in the late stages of infection, these fibrous layers gradually thicken, weakening the effect of immune cells on the lesions. *E. multilocularis* can achieve a certain balance between pro-inflammatory and anti-inflammatory through the formation of an “immune tolerance” microenvironment, maintain immune homeostasis, and help itself survive in the host [3].

As all we know, IL-10 is an anti-inflammatory cytokine, which plays an important role in the formation of immune tolerance in a variety of diseases, including parasitic diseases [4–7]. Regulatory B cells (Bregs)

are a special subset of B cells that have immune regulation or immunosuppressive functions, and play an important role in peripheral immune tolerance. The activity of Breg is mainly attributable to the expression of IL-10, but also uses additional regulatory mechanisms such as TGF-β, FasL, IL-35 and TIGIT [8, 9]. T cells immunoglobulin domain and mucin domain protein-1 (TIM-1) is a transmembrane glycoprotein that has been identified as a member of the TIM family of genes that regulate immune response. TIM-1 is first found to be expressed on T cells and dendritic cells, and plays an important role in regulating important cell functions [10]. Recently, it has also been found that TIM-1 is expressed in B cells. The function of TIM-1$^+$B cells requires the expression of TIM-1, which is essential for maintaining autoimmune tolerance and limiting tissue inflammation. The vast majority of TIM-1$^+$B cells produce IL-10. Compared with all other B cell subsets, the expression of IL-10 is increased by 8–20 times and it accounts for over 70% of all B cells that produce IL-10 [11]. Therefore, TIM-1$^+$B cells have become a new focus of research on immune tolerance.

One of the typical characteristics of AE is the vesicles wrapped by a layer of collagen fibers. The increasing volume and number will seriously affect the normal physiological function of the host. If it is not inhibited, it will inevitably cause the death of the host. IL-10 is believed to promote fibrosis, reduce the secretion of pro-inflammatory cytokines, and accelerate the resolution of inflammation. In view of the fact that Breg and TIM-1$^+$B cells show strong regulatory abilities and IL-10 secretion in other infectious diseases, we want to know whether they are also involved in the pathogenesis of AE. We propose a study to observe the role of Breg and TIM-1$^+$B cells in liver fibrosis caused by AE.

Material And Methods

Mice

Pathogen-free female BALB/c mice (6-8 weeks old) were purchased from the First Affiliated Hospital of Xinjiang Medical University Experimental Animal Science Research Department. All animal procedures were approved by the Animal Care and Use Committee and the Ethical Committee of First Affiliated Hospital of Xinjiang Medical University (Approving Number: 20170214-106).

E. multilocularis infection mouse model

Fourteen BALB/c mice were randomly divided into 2 groups, the infection group (7) and the control group (7). *E. multilocularis* protoscoleces (PSCs) were obtained from intraperitoneal lesions maintained in BALB/c. Briefly, the parasites were washed several times using phosphate buffered saline (PBS) containing 1000 mg/ml penicillin and 1000 U/ml streptomycin. Finally, counted using a microscope and adjusted to the appropriate parasite concentration before injection [12]. The concentration is 2000 PSCs/ml. The injection method is intraperitoneal injection. The mice in infection group were injected with 1 ml of PSCs sediment, and the mice in the control group were injected with the same amount of saline.

They were maintained in an air-conditioned animal room with a 12-hour light/dark cycle, and provided with rodent chow and water.

Specimen collection

Mice were sacrificed at experimental timepoints (180 days after model was established) using euthanasia, which was approved by Institutional Animal Care and Use Committee. Specifically, mice were intraperitoneally (left lower quadrant) injected with 5% chloral hydrate 0.15–0.20 ml/mouse through medical syringe needle, and peripheral blood was collected by retro-orbital bleeding after successfully anesthetized; then, whole liver and spleen samples were obtained surgically; at last, mice were euthanized using cervical dislocation [13].

Cell culture

The spleen was grinded and used mouse spleen lymphocyte extraction kit to separate lymphocytes. The separated lymphocytes were cultured in RPMI 1640 medium supplemented with 10% FBS at a density of 2×10^6 cells/ml in a 24-well plate. LPS with a final concentration of 10 μg/ml was added to each well, and cultured at 37℃ and 5% CO_2 for 20h. 2μl of Leukocyte Activation Cocktail was added to each well for 4h. Cells were collected in 1.5ml EP tube and waited for flow cytometry to detect the cells.

Flow cytometry

The collecting cells stained with appropriate concentrations of fluorochrome-conjugated antibodies at 4℃ for 30 min, and then the cells were washed with PBS solution. The antibodies included anti CD1d-PE, anti CD5-PECy5, anti CD19-FITC, anti TIM-1-PE. The remaining cells were fixed and permeabilized using eBioscience™ fixation/permeabilization concentrate and permeabilization buffer according to the manufacture's description. Remaining cells stained with appropriate concentrations of fluorochrome-conjugated antibody (anti IL-10-APC) at 4℃ for 30 min, and then the cells were washed with PBS solution. The cells were suspended in 300μl of PBS solution and then acquired using the DxFLEX.

Histopathological analysis

The liver samples were fixed with 4% paraformaldehyde, dehydrated, and embedded in paraffin. Sections (4μm thick) from embedding tissue were prepared and stained with hematoxylin, eosin and Masson staining kit.

Immunohistochemistry and Immunofluorescence analysis

For the IHC analysis, sections (4μm thick) from embedding tissue were prepared. Then, the sections were incubated with a primary antibody against mouse Desmin, TIM-1 and IL-10. Immunoreactive proteins were visualized using the appropriate anti-IgG secondary antibodies labeled with horseradish peroxidase and the chromogen 3'-diaminobenzidine (DAB) as a substrate.

For the IF analysis, paraffin-embedded sections were stained with rabbit anti CD20 and rat anti TIM-4 antibodies, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) and Goat Anti-Rat IgG H&L (Alexa Fluor® 594). Positive cells were detected by confocal microscopy.

RNA isolation and qRT-PCR

Total RNA was extracted from whole liver tissues using Trizol reagent and subsequently reversely transcribed into cDNA using a PrimeScript RT reagent kit according to the manufacturer's instructions. The cDNA was subjected to real-time PCR using a SYBR Premix Ex Taq II kit and an Applied Biosystems 7500 Fast Real-Time PCR System. GAPDH was used as an internal control. The results were calculated by the $2^{-\triangle\triangle Ct}$ method. The primers used for qRT-PCR were listed below:

IL-10: (F) GCTGGACAACATACTGCTAACC (R) ATTTCCGATAAGGCTTGG CAA

GAPDH: (F) AACTTTGGCATTGTGGAAGG (R) CACATTGGGGGTAGGAA CAC

Enzyme-linked Immunosorbent Assay (ELISA)

According to the manufacturer's instructions, IL-10 in serum was analyzed with an ELISA kit. Data was detected with a microplate reader and analyzed thereafter.

Statistical analysis

The quantitative analysis of morphology results was carried out by ImageJ software; Statistical analysis and mapping were carried out by GraphPad Prism V8.0 software. The results are expressed as the mean ± SD. The independent-sample t-test is used for the comparison between the two groups of means, the one-way analysis of variance is used for the comparison of the means between multiple groups. $P<0.05$ indicates that the difference is significant.

Result

In the late stage of AE, severe liver fibrosis will be formed with a large number of activated HSC and TIM-1 $^{+}$ cell infiltration

First, we observed by HE staining that there was no obvious abnormality in the liver of the mice in the control group. The normal liver lobule structure of the mice in the infection group was destroyed, hepatocytes were degenerated and necrotic, hepatic sinusoids were dilated. There is a large amount of inflammatory cells around the lesion (Fig. 1A, B). Then, we used Masson staining to compare the situation of fibrosis in the liver of the two groups of mice. The results showed that the fibrosis area in the infection group was significantly higher than that in the control group (Fig. 2A, B, C). Desmin is one of the markers of HSC activation [14]. Our IHC results showed that there was a large amount of Desmin expression around the liver lesions in the infection group, and it was significantly higher than that in the control group (Fig. 3A, B). Suggesting that there may be activated HSC near the lesion, and the activated HSC contribute to the formation of extracellular matrix, thereby promoting the progression of liver fibrosis. In mice, TIM-1 is mainly expressed in Th2 cells, B cells, invariant natural killer T (iNKT) cells and kidney tubular epithelial cells [15]. Our IHC results showed that there was a large amount of TIM-1^+ cell in the liver lesions in the infection group, which was significantly different from the control group, suggesting that TIM-1^+ cells were involved in the progression of AE (Fig. 3A, C).

Immune tolerance-related cytokine IL-10 is up-regulated in the late stage of AE

The previous results of this study showed that TIM-1^+ cells are abundantly expressed around the lesion, and most of these cells are related to immune tolerance. Therefore, we use a variety of methods to detect cytokine IL-10 that is inseparable from the formation of immune tolerance. The results of IHC showed that the expression of IL-10 increased around the liver lesions of the infection group. It was significantly different from the control group (Fig. 4A, B). The qRT-PCR results showed that compared with the control group, the liver IL-10 mRNA of the infection group was significantly up-regulated (Fig. 4C). The Elisa results also showed that the IL-10 content in the serum of the infection group was significantly higher than that of the control group (Fig. 4D). Our results imply that IL-10 may play an indispensable role in liver fibrosis caused by AE.

CD19 $^+$ TIM-1 $^+$ cells are more efficient of producing IL-10 than $CD19^+CD5^+CD1d^{hi}$ cells

We have previously verified that IL-10 is significantly increased in AE lesions. However, which cells are the sources of these IL-10? Then, we used flow cytometry to detect the level and function of $CD19^+$TIM-1^+ cells and $CD19^+CD5^+CD1d^{hi}$ cells in the spleen of the two groups of mice. The results showed that $CD19^+$ cells, $CD19^+$TIM-1^+ cells, $CD19^+$IL-10^+ cells and $CD19^+CD5^+CD1d^{hi}$ cells in the infection group were significantly higher than those in the control group (Fig. 5A, B, C, D, F, G). The expression of TIM-1 in IL-10-producing B cells in the infection group was significantly higher than that in the control group (Fig. 5A, B, E). The proportion of $CD19^+CD5^+CD1d^{hi}$IL-10^+ cells and $CD19^+$TIM-1^+IL-10^+ cells in the infection group

were significantly higher than control group. In the infection group, the proportion of IL-10 producing cells in $CD19^+TIM\text{-}1^+$ cells was significantly higher than that in $CD19^+CD5^+CD1d^{hi}$ cells (Fig. 5A, B, C, D, H). The above results indicate that in the late stage of AE, $CD19^+CD5^+CD1d^{hi}$ cells and $TIM\text{-}1^+$B cells are up-regulated. The ability of them to produce IL-10 is higher than that of control group, but the ability of $TIM\text{-}1^+$B cells is stronger. It suggests that the increased $TIM\text{-}1^+$B cells may be a more efficient IL-10 producer in AE.

TIM-1 $^+$ B cells are recruited to the lesion by binding to the ligand TIM-4

We have verified that in the spleen at the late stage of AE, there will be more $TIM\text{-}1^+$B cells that producing IL-10. Will these cells reach the lesion? Finally, we performed IF detection. Our confocal microscopy results showed that $TIM\text{-}4^+$ cells and B cells were located in the same region (Fig. 6). It suggests that the $TIM\text{-}1^+$B cells may be recruited into the lesion through TIM-1/TIM-4 signaling in AE.

Discussion

Alveolar echinococcosis as a tropical disease neglected by the World Health Organization is prevalent all over the world. In Asia, China is still one of the regions with the highest prevalence, especially in western China. Therefore, it is urgent to find an effective treatment for AE [1, 16]. In the persistent infection of *E. multilocularis*, the formation of collagen fibrous layer is one of the typical pathological changes. Furthermore, based on the morphological and immunohistochemical data, the lesion is characterized by an outer layer consisting of T and B cells [17]. It shows that B cells may be involved in the formation of liver fibrosis caused by echinococcosis. In human and animal models, the continuous parasitic infection and the occurrence of tumors are both heavily involved in regulatory B cells. Their role is inseparable from an important cytokine IL-10 [18–21].

In recent years, more and more researchers have noticed a new subset of B cells that secrete IL-10 more efficiently, $TIM\text{-}1^+$B cells. Examination of $TIM\text{-}1^+$ B cells revealed a 20- to 25-fold enrichment for IL-10 compared to $TIM\text{-}1^-$ B cells [9]. TIM-1 binds to phosphatidylserine, which is flipped to the outer leaflet of apoptotic cell membranes, conveying phagocytosis by macrophages and IL-10 expression by B cells. Therefore, some researchers suggest that TIM-1 is one of the markers for the identification of Breg subset [9, 22]. Studies have shown that mouse $TIM\text{-}1^+$B cells can suppress Th1 response *in vivo* and promote Th2 and Treg responses in an allograft transplantation setting [23]. In the experimental autoimmune encephalomyelitis model, $TIM\text{-}1^+$B cells inhibit Th1 and Th17 responses *in vivo* [24]. Additionally, TIM-1 signaling is essential for regulating the balance between “regulatory” and proinflammatory B cells in order to maintain self-tolerance in tissues including the CNS [25]. Despite the above inspiring research results, it is still unclear that the exact mechanism of TIM-1’s immune regulation. Until now, there have

been few studies that the relationship between TIM-1$^+$B cells and AE. Therefore, we made this research work.

First, we made a model by intraperitoneal injection of the PSCs to simulate a process termed “secondary” echinococcosis. After 180 days of infection, we used HE staining and Masson staining to observe the livers of the mice in the control and infection group. The results showed that the area of inflammatory cell infiltration and fibrosis in the liver of the infection group was significantly higher than that of the control. Subsequently, we detected the expression of Desmin and TIM-1, which level were also significantly higher than the control. It suggests that in the liver fibrosis caused by AE, activated HSC and TIM-1$^+$ cells may be involved in the formation's process of fibrosis. Finally, we detected the expression of IL-10 in the liver and peripheral blood, and found that the infection group was also significantly higher than the control. It shows that IL-10 may help the formation of liver fibrosis in the late stage of AE.

In many diseases with immune tolerance, IL-10 has always been one of the important factors influencing the balance of immune status. It has a wide range of sources and Breg is one of its sources. The spleen is the largest reservoir for Breg and is the focus of most murine Breg studies [26]. In this study, our flow cytometry results showed that TIM-1$^+$B cells in the spleen of mice in the infection group had a higher proportion of IL-10 producing cells than CD19$^+$CD5$^+$CD1d^{hi} cells, and both were higher than those in the control.

TIM-4, the natural ligand for TIM-1, was mainly expressed by CD11b$^+$ myeloid cells [27],the confocal microscopy results showed that TIM-4$^+$ myeloid cells and B cells were located in the same region, which suggests that myeloid cells could promote TIM-1$^+$B cells to produce IL-10 through TIM-1/TIM-4 signaling. Therefore, this may be one of reasons for the high expression of IL-10 in the AE lesion and the formation of fibrosis in the local area.

In mice, TIM-1 has been reported to be an important receptor, which can induce and exert the functions of Breg cells, maintain transplantation immune tolerance and prevent autoimmunity. There are research findings that TIM-1 ligation in B cells induces IL-10$^+$ Breg which are required to prolong graft survival [11]. In addition, the TIM-1 signal is necessary to inhibit the production of pro-inflammatory cytokines by Breg. The interaction between TIM-1 and TIM-4 plays a crucial role in Th2 immune response [28].

The results of this study suggest that in AE, TIM-1$^+$B cells and CD19$^+$CD5$^+$CD1d^{hi} cells are both IL-10 producers. The source of the high expression of IL-10 in the lesion may be produced by the recruitment of TIM-1$^+$B cells with high IL-10 production through the TIM-1/TIM-4 signaling.

In summary, we prove that TIM-1$^+$B cells are essential for maintaining immune tolerance in AE. The regulatory function of TIM-1$^+$B cells in infection and transplantation has also been determined [23, 29, 30]. Thus, further understanding TIM-1$^+$B cells and their regulatory mechanisms would be valuable for treating immune-related diseases by selectively enhancing or inhibiting the B cell activity and/or their regulatory mechanisms.

Conclusions

In liver fibrosis caused by AE, TIM-1^{+}B cells and CD19^{+}CD5^{+}CD1d^{hi} cells are both producers of IL-10. TIM-1^{+}B cells can be recruited to the lesion, which are more efficient IL-10 producer.

Declarations

Ethics approval and consent to participate

All animal procedures were approved by the Animal Care and Use Committee and the Ethical Committee of First Affiliated Hospital of Xinjiang Medical University (Approving Number: 20170214-106). All surgery was performed under chloral hydrate anesthesia, and all efforts were made to minimize suffering.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the National Natural Science Foundation (82060372, 81760372). Scientific Research Project of Science Department of Xinjiang Autonomous Region (2020E0277) and State Key Laboratory of Pathogenesis, Prevention and Treatment of High Incidence Diseases in Central Asia Fund (SKL-HIDCA-2020-YG3SKL-HIDCA-2020-BC5). The funding bodies had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Authors' contributions

Prof. MXM and CJ conceived and designed the study. LB and QXW drafted the manuscript, MXM and LB critically revised manuscript. LB, JT, LYM, SJY, CXL, conducted experimental work. QXW, LJ, AM interpreted data. All the authors read and approved the final manuscript.

Acknowledgements

Bin Li especially wants to thank Huimin Zhu. The patience, encouragement and care from her have given him powerful spiritual support over the past years.

References

1. Baumann S, Shi R, Liu W, Bao H, Schmidberger J, Kratzer W, Li W, interdisciplinary Echinococcosis Working Group U: Worldwide literature on epidemiology of human alveolar echinococcosis: a systematic review of research published in the twenty-first century. Infection 2019, 47(5):703–727.
2. Wen H, Vuitton L, Tuxun T, Li J, Vuitton DA, Zhang W, McManus DP: Echinococcosis: Advances in the 21st Century. Clin Microbiol Rev 2019, 32(2).
3. Zhang C, Lin R, Li Z, Yang S, Bi X, Wang H, Aini A, Zhang N, Abulizi A, Sun C et al: Immune Exhaustion of T Cells in Alveolar Echinococcosis Patients and Its Reversal by Blocking Checkpoint Receptor TIGIT in a Murine Model. Hepatology 2020, 71(4):1297–1315.
4. Wynn TA: Type 2 cytokines: mechanisms and therapeutic strategies. Nat Rev Immunol 2015, 15(5):271–282.
5. Sziksz E, Pap D, Lippai R, Beres NJ, Fekete A, Szabo AJ, Vannay A: Fibrosis Related Inflammatory Mediators: Role of the IL-10 Cytokine Family. Mediators Inflamm 2015, 2015:764641.
6. Cerqueira C, Manfroi B, Fillatreau S: IL-10-producing regulatory B cells and plasmocytes: Molecular mechanisms and disease relevance. Semin Immunol 2019, 44:101323.
7. Boonpiyathad T, Satitsuksanoa P, Akdis M, Akdis CA: Il-10 producing T and B cells in allergy. Semin Immunol 2019, 44:101326.
8. Cai X, Zhang L, Wei W: Regulatory B cells in inflammatory diseases and tumor. Int Immunopharmacol 2019, 67:281–286.
9. Cherukuri A, Mohib K, Rothstein DM: Regulatory B cells: TIM-1, transplant tolerance, and rejection. Immunol Rev 2021, 299(1):31–44.
10. Xiao S, Brooks CR, Sobel RA, Kuchroo VK: Tim-1 is essential for induction and maintenance of IL-10 in regulatory B cells and their regulation of tissue inflammation. J Immunol 2015, 194(4):1602–1608.
11. Yeung MY, Ding Q, Brooks CR, Xiao S, Workman CJ, Vignali DA, Ueno T, Padera RF, Kuchroo VK, Najafian N et al: TIM-1 signaling is required for maintenance and induction of regulatory B cells. Am J Transplant 2015, 15(4):942–953.
12. Zhang C, Shao Y, Yang S, Bi X, Li L, Wang H, Yang N, Li Z, Sun C, Li L et al: T-cell tolerance and exhaustion in the clearance of Echinococcus multilocularis: role of inoculum size in a quantitative hepatic experimental model. Sci Rep 2017, 7(1):11153.
13. Abulizi A, Shao Y, Aji T, Li Z, Zhang C, Aini A, Wang H, Tuxun T, Li L, Zhang N et al: Echinococcus multilocularis inoculation induces NK cell functional decrease through high expression of NKG2A in

C57BL/6 mice. BMC Infect Dis 2019, 19(1):792.

14. Liu Y, Tian F, Shan J, Gao J, Li B, Lv J, Zhou X, Cai X, Wen H, Ma X: Kupffer Cells: Important Participant of Hepatic Alveolar Echinococcosis. Front Cell Infect Microbiol 2020, 10:8.
15. Freeman GJ, Casasnovas JM, Umetsu DT, DeKruyff RH: TIM genes: a family of cell surface phosphatidylserine receptors that regulate innate and adaptive immunity. Immunol Rev 2010, 235(1):172–189.
16. Wang Q, Yang L, Wang Y, Zhang GJ, Zhong B, Wu WP, Zheng CJ, Liao S, Yu WJ, He W et al: Disease burden of echinococcosis in Tibetan communities-A significant public health issue in an underdeveloped region of western China. Acta Trop 2020, 203:105283.
17. Ricken FJ, Nell J, Gruner B, Schmidberger J, Kaltenbach T, Kratzer W, Hillenbrand A, Henne-Bruns D, Deplazes P, Moller P et al: Albendazole increases the inflammatory response and the amount of Em2-positive small particles of Echinococcus multilocularis (spems) in human hepatic alveolar echinococcosis lesions. PLoS Negl Trop Dis 2017, 11(5):e0005636.
18. Han X, Yang J, Zhang Y, Zhang Y, Cao H, Cao Y, Qi Z: Potential Role for Regulatory B Cells as a Major Source of Interleukin-10 in Spleen from Plasmodium chabaudi-Infected Mice. Infect Immun 2018, 86(5).
19. Ritter M, Osei-Mensah J, Debrah LB, Kwarteng A, Mubarik Y, Debrah AY, Pfarr K, Hoerauf A, Layland LE: Wuchereria bancrofti-infected individuals harbor distinct IL-10-producing regulatory B and T cell subsets which are affected by anti-filarial treatment. PLoS Negl Trop Dis 2019, 13(5):e0007436.
20. Chen F, Wu W, Jin L, Millman A, Palma M, El-Naccache DW, Lothstein KE, Dong C, Edelblum KL, Gause WC: B Cells Produce the Tissue-Protective Protein RELMalpha during Helminth Infection, which Inhibits IL-17 Expression and Limits Emphysema. Cell Rep 2018, 25(10):2775–2783 e2773.
21. Rong HM, Li T, Zhang C, Wang D, Hu Y, Zhai K, Shi HZ, Tong ZH: IL-10-producing B cells regulate Th1/Th17-cell immune responses in Pneumocystis pneumonia. Am J Physiol Lung Cell Mol Physiol 2019, 316(1):L291-L301.
22. Aravena O, Ferrier A, Menon M, Mauri C, Aguillon JC, Soto L, Catalan D: TIM-1 defines a human regulatory B cell population that is altered in frequency and function in systemic sclerosis patients. Arthritis Res Ther 2017, 19(1):8.
23. Ding Q, Yeung M, Camirand G, Zeng Q, Akiba H, Yagita H, Chalasani G, Sayegh MH, Najafian N, Rothstein DM: Regulatory B cells are identified by expression of TIM-1 and can be induced through TIM-1 ligation to promote tolerance in mice. J Clin Invest 2011, 121(9):3645–3656.
24. Xiao S, Brooks CR, Zhu C, Wu C, Sweere JM, Petecka S, Yeste A, Quintana FJ, Ichimura T, Sobel RA et al: Defect in regulatory B-cell function and development of systemic autoimmunity in T-cell Ig mucin 1 (Tim-1) mucin domain-mutant mice. Proc Natl Acad Sci U S A 2012, 109(30):12105–12110.
25. Xiao S, Bod L, Pochet N, Kota SB, Hu D, Madi A, Kilpatrick J, Shi J, Ho A, Zhang H et al: Checkpoint Receptor TIGIT Expressed on Tim-1(+) B Cells Regulates Tissue Inflammation. Cell Rep 2020, 32(2):107892.

26. DiLillo DJ, Matsushita T, Tedder TF: B10 cells and regulatory B cells balance immune responses during inflammation, autoimmunity, and cancer. Ann N Y Acad Sci 2010, 1183:38–57.
27. Ye L, Zhang Q, Cheng Y, Chen X, Wang G, Shi M, Zhang T, Cao Y, Pan H, Zhang L et al: Tumor-derived exosomal HMGB1 fosters hepatocellular carcinoma immune evasion by promoting TIM-1(+) regulatory B cell expansion. J Immunother Cancer 2018, 6(1):145.
28. Meyers JH, Chakravarti S, Schlesinger D, Illes Z, Waldner H, Umetsu SE, Kenny J, Zheng XX, Umetsu DT, DeKruyff RH et al: TIM-4 is the ligand for TIM-1, and the TIM-1-TIM-4 interaction regulates T cell proliferation. Nat Immunol 2005, 6(5):455–464.
29. Liu J, Zhan W, Kim CJ, Clayton K, Zhao H, Lee E, Cao JC, Ziegler B, Gregor A, Yue FY et al: IL-10-producing B cells are induced early in HIV-1 infection and suppress HIV-1-specific T cell responses. PLoS One 2014, 9(2):e89236.
30. Mao H, Pan F, Wu Z, Wang Z, Zhou Y, Zhang P, Gou M, Dai G: Colorectal tumors are enriched with regulatory plasmablasts with capacity in suppressing T cell inflammation. Int Immunopharmacol 2017, 49:95–101.

Figures

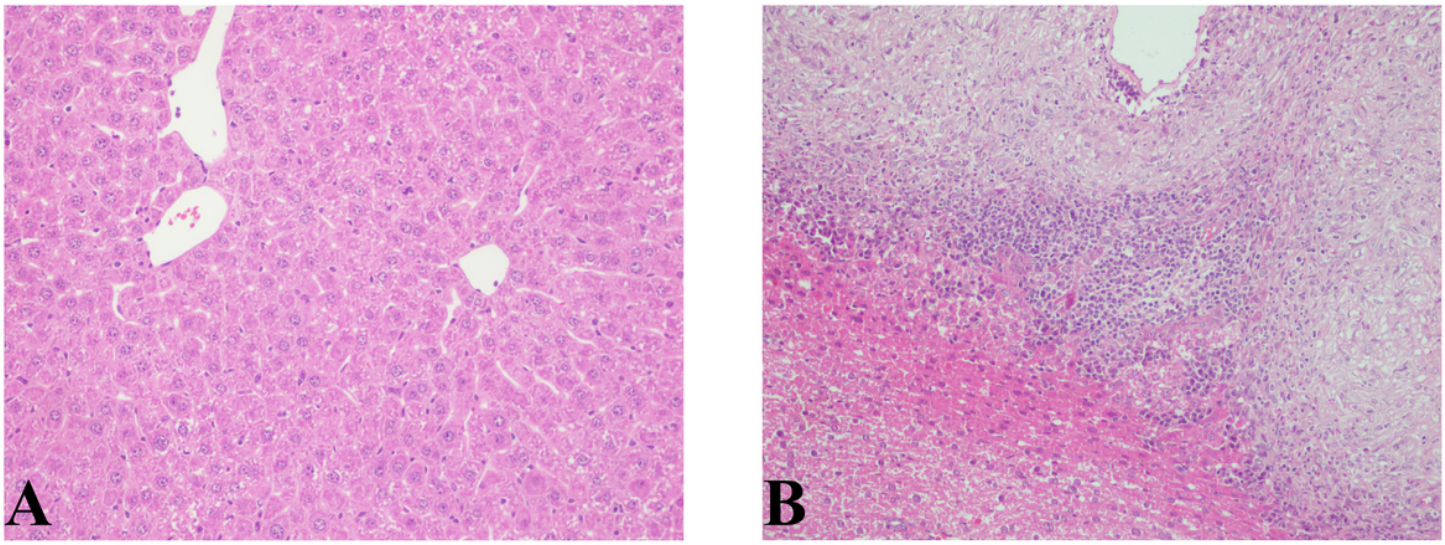

Figure 1

Representative HE images in liver tissue are shown (200×). (A) Control (B) Infection

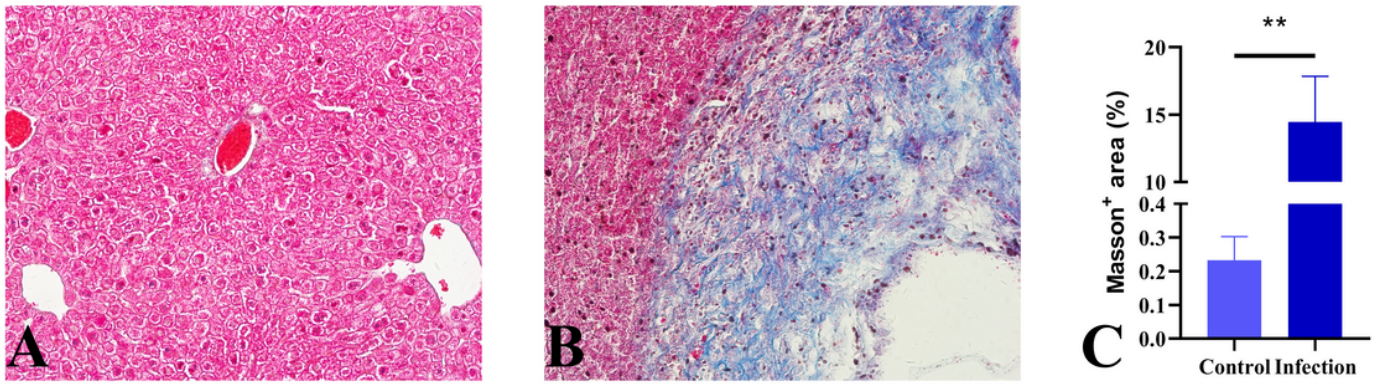

Figure 2

Representative Masson images in liver tissue are shown (200×). (A) Control (B) Infection (C) The positive areas of Masson staining in (A) and (B) were quantitatively compared. Bars = means ± SD, n = 7. *p<0.05, **p<0.01.

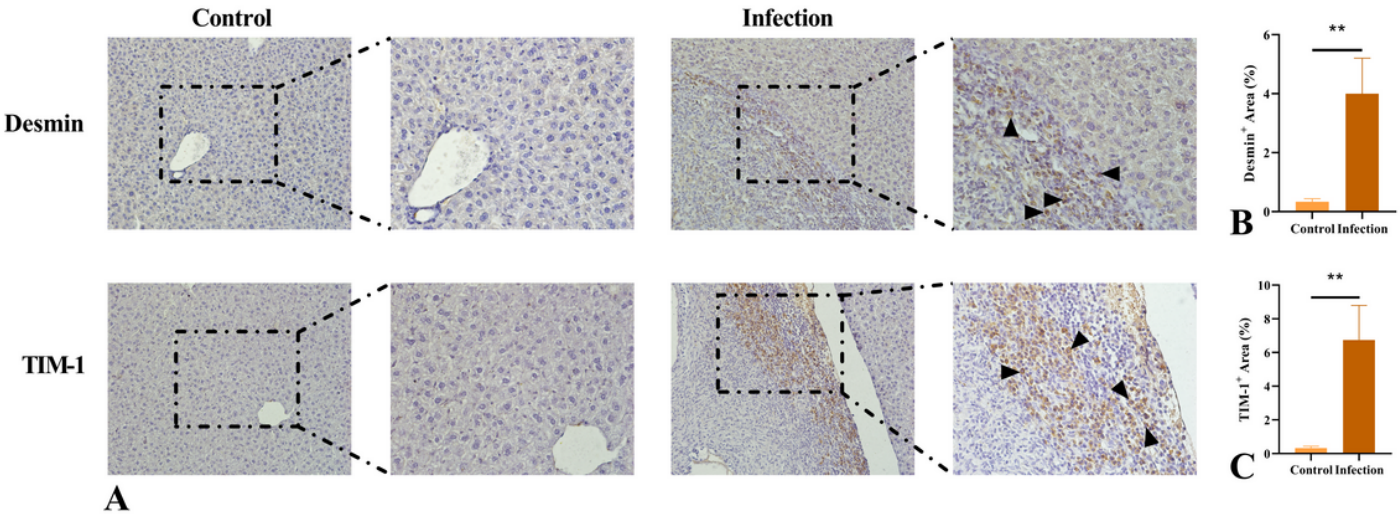

Figure 3

There are numerous activated HSC and TIM-1+ cells in the lesions of alveolar echinococcosis. (A) Representative immunohistochemistry images of Desmin and TIM-1 in liver tissue are shown (left panel 200×, enlarged 400× on the right panel). The brown grains indicated the positively stained regions. Arrowheads indicate representative positive cells. (B, C) The Desmin-positive areas (B) and the TIM-1-positive areas (C) in (A) were quantitatively compared. Bars = means ± SD, n = 7. *p<0.05, **p<0.01.

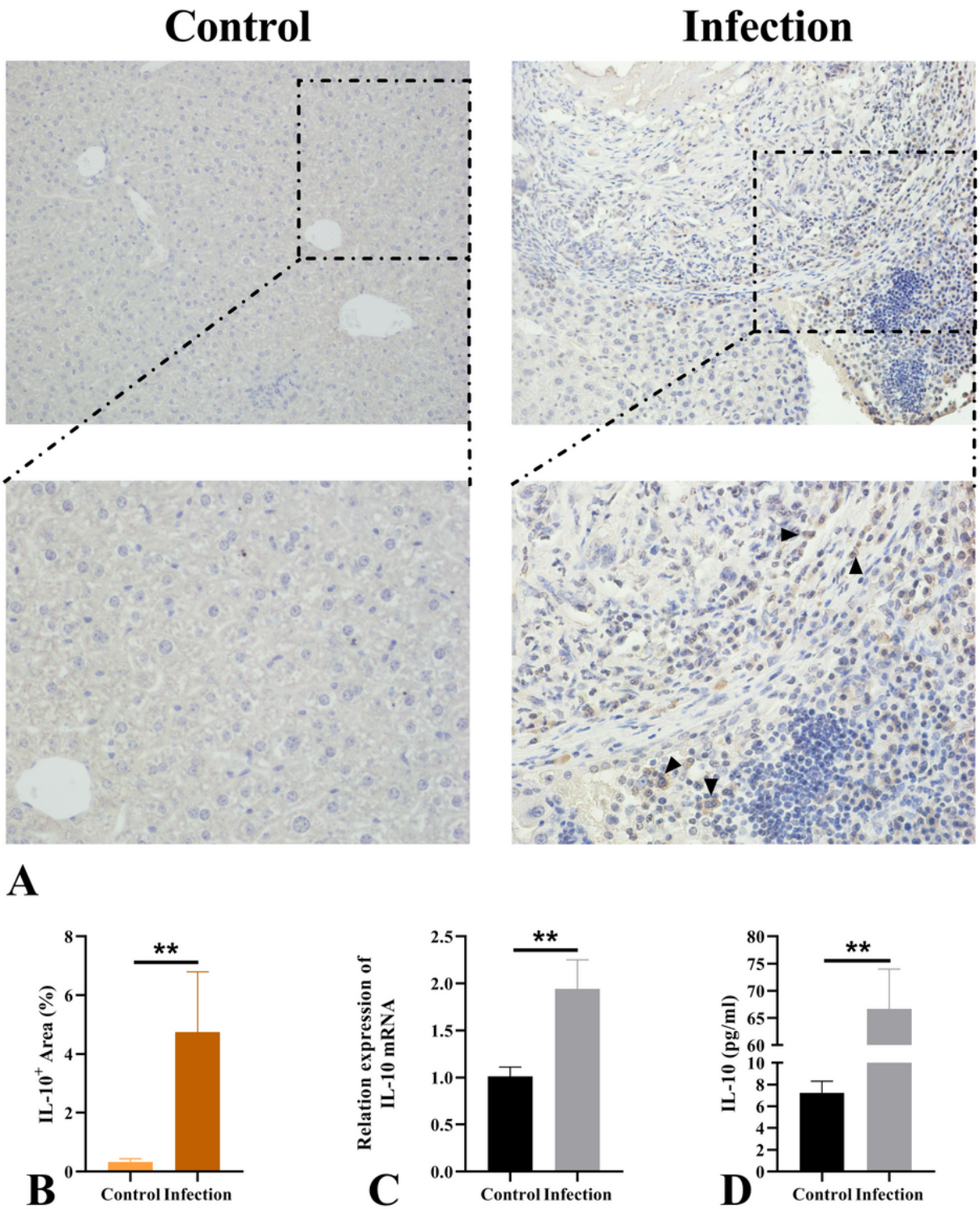

Figure 4

Up-regulated expression of IL-10 in the lesions and serum of alveolar echinococcosis. (A) Representative immunohistochemistry images of Il-10 in liver tissue are shown (upper panel 200×, enlarged 400× on the lower panel). The brown grains indicated the positively stained regions. Arrowheads indicate representative positive cells. (B) The IL-10-positive areas in (A) were quantitatively compared. (C) Total liver RNA was extracted, and the expression of IL-10 was determined by quantitative reverse transcription

(qRT)-PCR. (D) The concentrations of IL-10 in serum were determined by Elisa. Bars = means ± SD, n = 7. *p<0.05, **p<0.01.

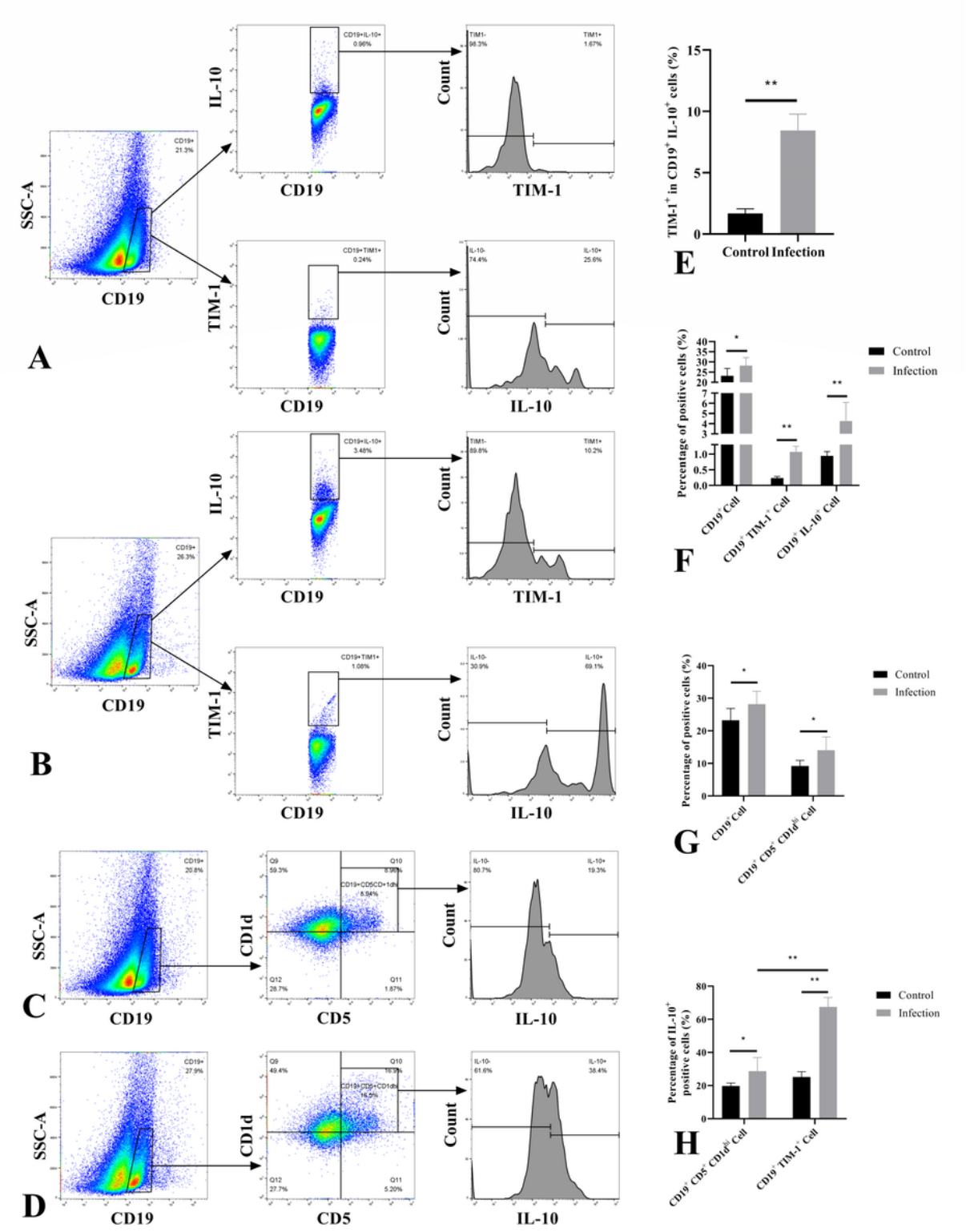

Figure 5

CD19+TIM-1+ cells are more efficient of producing IL-10 than CD19+CD5+CD1dhi cells in the spleen of alveolar echinococcosis. (A, B, C, D) Representative images of Spleen lymphocytes were analyzed by fluorescence-activated cell sorting (FACS) after staining with anti-CD19, anti-CD5, anti-CD1d, anti-TIM-1

anti-IL-10. (E) The percentage of TIM-1+ cells in CD19+IL-10+ cells in (A, B) was determined and quantitatively compared. (F) The percentage of CD19+ cells, CD19+TIM-1+ cells, CD19+IL-10+ cells in (A, B) was determined and quantitatively compared. (G) The percentage of CD19+CD5+CD1dhi cells in (C, D) was determined and quantitatively compared. (H) The percentage of IL-10+ cells in both CD19+CD5+CD1dhi cells and CD19+TIM-1+ cells in (A, B, C, D) was determined and quantitatively compared. Bars = means ± SD, n = 7. *p<0.05, **p<0.01.

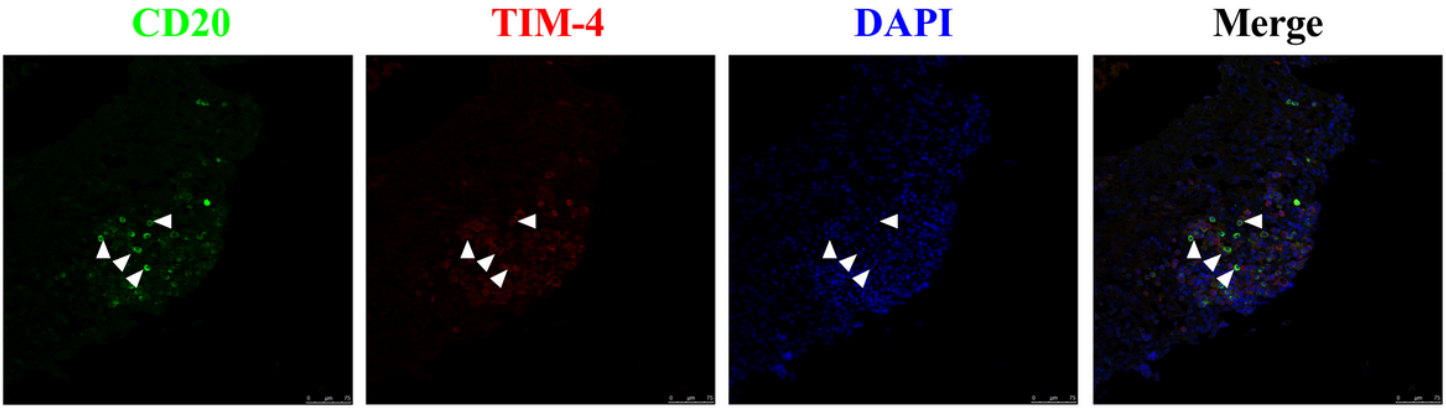

Figure 6

Representative immunofluorescence images of CD20 (green), TIM-4 (red) and nuclear staining with DAPI (blue) in infection group's liver tissue are shown (200×).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- NC3RsARRIVEGuidelinesChecklist.pdf