Original Article

Iranian Journal of Immunology

https://iji.sums.ac.ir

# The Proportion of Vδ1T Cells in Peripheral Blood Is Correlated with Prognosis of Sepsis

Fawei Yuan[1], Huan Yin[2], Juan Tan[3], Kun Zheng[1]*, Xiping Mei[1], Lixue Yuan[1]

[1]Department of Intensive Care Unit, Huangshi Central Hospital of Edong Healthcare Group (Affiliated Hospital of Hubei Polytechnic University), Hubei Key Laboratory of Kideny Disease Pathogenisis and Intervention, Huangshi 435000, Hubei Province, China; [2]Department of Neonatal, Huangshi Maternal and Child Health Hospital of Edong Healthcare Group, Huangshi 435000, Hubei Province, China; [3]Department of Emergency, Huangshi Central Hospital of Edong Healthcare Group (Affiliated Hospital of Hubei Polytechnic University), Hubei Key Laboratory of Kideny Disease Pathogenisis and Intervention, Huangshi 435000, Hubei Province, China

## ABSTRACT

**Background:** Sepsis is a serious condition with a high mortality rate, and septic patients often have organ dysfunction, low tissue perfusion and hypoxia, lactic acidosis, oliguria, or functional brain changes.

**Objective:** To observe the number and the function of Vδ1T cells in peripheral blood of septic patients, to analyze the clinical significance of detecting Vδ1T cells, and to clarify the correlation of their presence with the prognosis of sepsis.

**Methods:** The basic data of the septic patients were recorded at admission. The immunosuppressive function–related molecules on the surface of Vδ1T cells were detected, and the immunosuppressive function of Vδ1T cells was also evaluated.

**Results:** Compared with the healthy controls, the proportion of Vδ1T cells in the blood of septic patients significantly decreased ($P<0.01$). The proportion of Vδ1T cells in septic patients correlated with the patients' condition ($P<0.05$). The expression of glucocorticoid-induced tumor necrosis factor receptor (GITR), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), and T-cell immunoglobulin and mucin domain-containing protein 3 (TIM-3) on the surface of Vδ1T cells in the blood of septic patients significantly increased ($P<0.01$). The increase of Vδ1T cells in septic patients had inhibitory effects on T cell proliferation and interferon (IFN)-γ secretion. These findings implied that the immunosuppression of Vδ1Tcells in the peripheral blood of septic patients was significantly higher than that of the healthy controls ($P<0.01$).

**Conclusion:** Changes in Vδ1T cells in septic patients were closely related to the patient's condition and prognosis.

**Keywords:** Sepsis, Peripheral blood, Vδ1T cells, Immunosuppression, Proliferation

*Corresponding author:
Kun Zheng,
Department of Intensive Care Unit, Huangshi Central Hospital of Edong Healthcare Group (Affiliated Hospital of Hubei Polytechnic University), Hubei Key Laboratory of Kideny Disease Pathogenisis and Intervention, No.141 Tianjin Road, Huangshi 435000, Hubei Province, China
**Tel:** +86 714 6286623
**Email:** kunzheng1990@126.com

*Cite this article as:*
Yuan F, Yin H, Tan J, Zheng K, Mei X, Yuan L. The Proportion of Vδ1T Cells in Peripheral Blood Is Correlated with Prognosis of Sepsis. *Iran J Immunol.* 2022; 19(3):232-242, doi: 10.22034/iji.2022.89256.1934.

Received: 07-12-2020
Revised: 23-07-2022
Accepted: 25-07-2022

## INTRODUCTION

Sepsis refers to the systemic inflammatory response caused by infection and diagnosed by the presence of bacteria or highly suspicious infections (1). Sepsis is a dangerous condition

with a high mortality rate, and septic patients often have organ dysfunction, low tissue perfusion, and hypoxia, lactic acidosis, oliguria, or functional brain changes (2, 3). Although a series of studies have been conducted on sepsis over the past 20 years, more than one-half of deaths in intensive care units (ICU) are still caused by septic shock and multiple organ dysfunction, while sepsis is still the main cause of death for non-cardiac patients in the ICU (4, 5). At present, there has been no fundamental breakthrough regarding the treatment of sepsis due to the unclear underlying mechanism. Therefore, studying the pathogenesis of sepsis can help us better understand this complex syndrome and discover a more effective treatment.

The immunosuppression that occurs with sepsis is also described as a compensatory anti-inflammatory response syndrome (CARS) (6). The immune response to sepsis is characterized by phagocytosis, antigen presentation, and apoptotic functions of dendritic cells, neutrophils, T cells and, B cells, and it is also accompanied by a large amount of secretion of anti-inflammatory cytokines, including interleukin (IL)-4, IL-10, etc. (7-9). Some researchers believe that the initial stage of inflammation after the onset of sepsis is associated with the presence of anti-inflammatory responses, so patients with sepsis may experience the inability to clear the pathogen or have a secondary infection (10). As the disease progresses, patients with sepsis experience an immunosuppressive state which may explain why most clinical trials involving anti-inflammatory strategies do not significantly improve the prognosis of patients with sepsis (11). It means that correcting the immunosuppressive state of patients with sepsis might be important for the treatment of this disease (12). In recent years, the functions of immunosuppressive cells including regulatory T cells have been proven to be involved in maintaining the immunosuppressive state in septic patients (12-16). According to the expression of T cell receptors (TCR), T cells are mainly divided into 2 subsets: αβ T cells and γδ T cells (17). γδ T cells are a kind of T lymphocytes expressing TCR consisting of γ chains and δ chains on their surfaces and are regarded as innate immune cells (18). γδT cells are divided into two cell subsets: Vδ1 T cells andVδ2T cells. Vδ1T cells are mainly distributed in epithelial-related lymphoid tissue, and Vδ2T cells are mainly distributed in peripheral blood (19, 20). Vδ1T cells and Vδ2T cells are involved in different effects. Vδ2 T cells mainly play a role in the immune surveillance of tumors and defense responses (21, 22), while Vδ1 T cells mainly show immunoregulatory functions (23). γδ T cells have become a hot topic of immunotherapy used for the treatment of cancers due to their unique characteristics (24). Of note, the immunosuppressive function of Vδ1T cells was confirmed more than 10 years ago. These cells have been studied to confirm their immunosuppressive function in patients with tumors and autoimmune diseases (25-27). However, the role of Vδ1T cells has been rarely reported in sepsis. Therefore, this study aimed to further clarify the immunosuppressive state of patients with sepsis experience, especially the role of Vδ1T cells that were closely related to the prognosis of septic patients.

## MATERIAL AND METHODS

*Case Collection*

From October 2016 to December 2017, we collected 40 cases of sepsis (mean age 47.3±10.6 years) from the ICU and 40 healthy controls (mean age 45.2±11.6 years) for regular check-ups in our hospital. All patients were eligible based on the diagnostic criteria for sepsis (28) that were established by the International Conference on Sepsis in Washington, DC, in December 2001. Informed consent was obtained from all participants.

*Peripheral Blood Mononuclear Cell (PBMCs) Separation*

The 15 mL peripheral blood of the healthy controls and septic patients were collected in

a sodium citrate anticoagulant tube (Thermo Fisher Scientific Inc., Waltham, MA, USA) in the early morning. The appropriate amount of lymphocyte separation fluid (Biolegend, CA, USA) was added to the centrifuge tube; then, the diluted peripheral blood was slowly added to the centrifuge tube, placed on the upper level of the lymphocyte separation solution, and centrifuged as 200 g for 20 min. The white membrane layer of the middle white lymphocytes was gently absorbed with a straw and inserted using a centrifuge into a 10-mL sterile RPMI-1640 medium (Gibco, New York, USA) at 180 g for 15 min. The sterile RPMI-1640 medium was discarded, and the cell suspension was suspended in a 10-mL sterile RPMI-1640 medium and centrifuged at 150g for 8min. PBMCs were obtained and used for the following experiments.

*Sorting of γδ T Cells and Vδ1 T Cells*

Sorting γδ T cells and Vδ1 T cells was performed according to the published study in the literature (29, 30). Briefly, PBMCs were cultured with 0.2 mL of RPMI-1640 medium containing 0.125μg of anti-TCR Vδ1 monoclonal antibody (each well) on a 48-well plastic culture plate in an incubator at 37°C with 5% $CO_2$ for 2 h. After PBMCs were resuspended with RPI-1640 medium adding 10% fetal bovine serum (FBS; Gibco, New York, USA) cells were added to a 48-well plate (1.0 mL per well) coated with anti-TCRVδ1 monoclonal antibody (Cell Signaling Technology, Inc., Danvers, MA, USA) and cultured. Following 2 weeks of culture, the Vδ1T cells with purity higher than 90% were sorted out by flow cytometry.

*Detection of Surface Immunosuppressive Molecules in Vδ1 T Cells*

In an Eppendorf tube, $1\times10^6$ PBMCs were obtained and mixed with 1 mL of the RPMI-1640 medium with 10% FBS (Gibco, New York, USA) for washing, and then centrifuged at 250 g for 8 min. The supernatant was abandoned, and the abovementioned operation was repeated. Subsequently, the cells were suspended in a 0.1 mL cell culture medium, and incubated with 3 μL of PE-anti-CD3antibody and 3 μL of FITC-anti-TCR Vδ1 antibody or3 μL of PE-anti-CD3 antibody and 3 μL of APC-anti-human GITR antibody/APC-anti-human CTLA-4 antibody/ Pecy5-anti-human TIM-3 antibody at 4°C for 30 min. After being washed twice with the RPMI-1640 medium containing 10% FBS, the cells were suspended in 0.1 mL of a 1% poly formaldehyde-fixed solution (Biolegend, CA, USA) for flow cytometry. All antibodies were purchased from Cell Signaling Technology, Inc., Danvers, MA, USA.

*CFSE Cell Proliferation Detection*

Naïve CD4 T cells were washed once with a serum-free 10-mL RPMI 1640 culture medium, and cultured with a CFSE staining solution (Invitrogen, Carlsbad, CA, USA) with a final concentration of5 mmol/L for 10 min in a 5% $CO_2$ incubator at 37°C. And then 5 mL of pre-chilled CFSE staining stop solution containing 5% cultures of 1640 medium was added to the cell and placed on ice for dyeing. After centrifuging at 400 g for 8 min and washing it once with a 10 mL RPMI 1640 culture medium, the cells were suspended in an RPMI-1640 complete medium. The T cells and naiveCD4 T cells (1:5 ratio) were added to a 48-hole plate and incubated for 5 days, and the cells were detected using flow cytometry.

*Detection of Cytokine Secretion by Vδ1T Cells in CD4 T Cells*

PBMCs were obtained using an aseptic sorting method, and Vδ1T cells and CD4 T cells with a purity greater than 90%were obtained using flow cytometry. Two cell types were added to the 48-hole plate using a 1:1 ratio of 1μg/mL of CD3antibody and 2 g/mL of CD28antibody (BD, New Jersey, USA). After incubating for 72 h at 37°C in a 5% $CO_2$ incubator, a 10-μL cell activation cocktail was incubated for 6h in 24-plates. Cells were collected, stained, and mixed with an immobilized cell membrane. After washing the cells with a permeable liquid twice, 5 μL of IFN-γ antibody (BD, New Jersey, USA) was

added at room temperature for 30 min. The cells were suspended twice in 0.2 mL poly formaldehyde-fixed solution at a concentration of 1% (Invitrogen, Carlsbad, CA, USA), and the cells were detected using flow cytometry.

*Statistical Analysis*

Statistical Product and Service Solutions (SPSS) version 18.0 (SPSS Inc., Chicago, IL, USA) was used to analyze the data. Counting data were expressed as percentages, and the measurement data were expressed with mean±standard deviation (SD). A t-test was used to compare the measurement data between the two groups. Comparison of experimental groups was evaluated using a one-way Analysis of Variance (ANOVA) analysis, followed by a Bonferroni analysis. A P value<0.05 was considered significant.

## RESULTS

*Characteristics of Patients*

As shown in Table 1, patients with sepsis were matched with the healthy controls in age and sex, and there were no significant differences between the two groups (P>0.05). Clinical data for patients with sepsis indicated that the sources of infection included lung infection (52.5%), abdomen infection (40.0%), and urinary tract infection (5.0%). Pathogens were gram-positive bacilli (47.5%), gram-negative bacilli (20.0%), and Fungus (32.5%). 20 patients died in ICU within 30 days after admission. One patient was transferred from ICU to the general ward and died of malignant arrhythmia on the 45th day after admission. One patient died of multiple organ failure on the 73rd day after admission. A total of 22 patients with sepsis died during their hospitalization, 20 of them died within 30 days, and the remaining 18 patients were discharged after improvement. During the 1-year follow-up, 5 of the 18 patients re-developed pulmonary infection, 2 died of respiratory failure, and the remaining 3 were discharged from the hospital. 3 of the 18 patients had chronic renal insufficiency treated with hemodialysis. 1 patient of the 18 patients developed chronic heart failure and pulmonary hypertension.

*Detection of Vδ1T Cell Ratio in the Blood of Septic Patients*

The proportion of Vδ1T cells in the peripheral blood of septic patients was measured (Figure 1). The proportion of

**Table 1. Clinical characteristics of the patients**

| Items | Health controls | Patients with sepsis |
|---|---|---|
| Number | 40 | 40 |
| Age (year) | 45.2±11.6 | 47.3±10.6 |
| Sex (male/female) | 20/20 | 21/19 |
| SOFA score | - | 11.3±4.4 |
| Source of infection | | |
| Lung | - | 21(52.5%) |
| Abdomen | - | 16(40.0%) |
| Urinary tract infection | - | 2(5.0%) |
| Pathogen | | |
| Gram-positive bacilli | - | 19(47.5%) |
| Gram-negative bacilli | - | 8(20%) |
| Fungus | - | 13(32.5%) |
| White blood cell ($\times10^9$/L) | - | 15.9±6.2 |
| PCT, ng/mL | - | 5.3±4.8 |
| Mechanical ventilation | - | 13(32.5%) |
| Renal transplantation | - | 9(22.5%) |
| ICU hospitalization (day) | - | 23.7±8.1 |
| Mortality (survival/death) | - | 55.0%(18/22) |

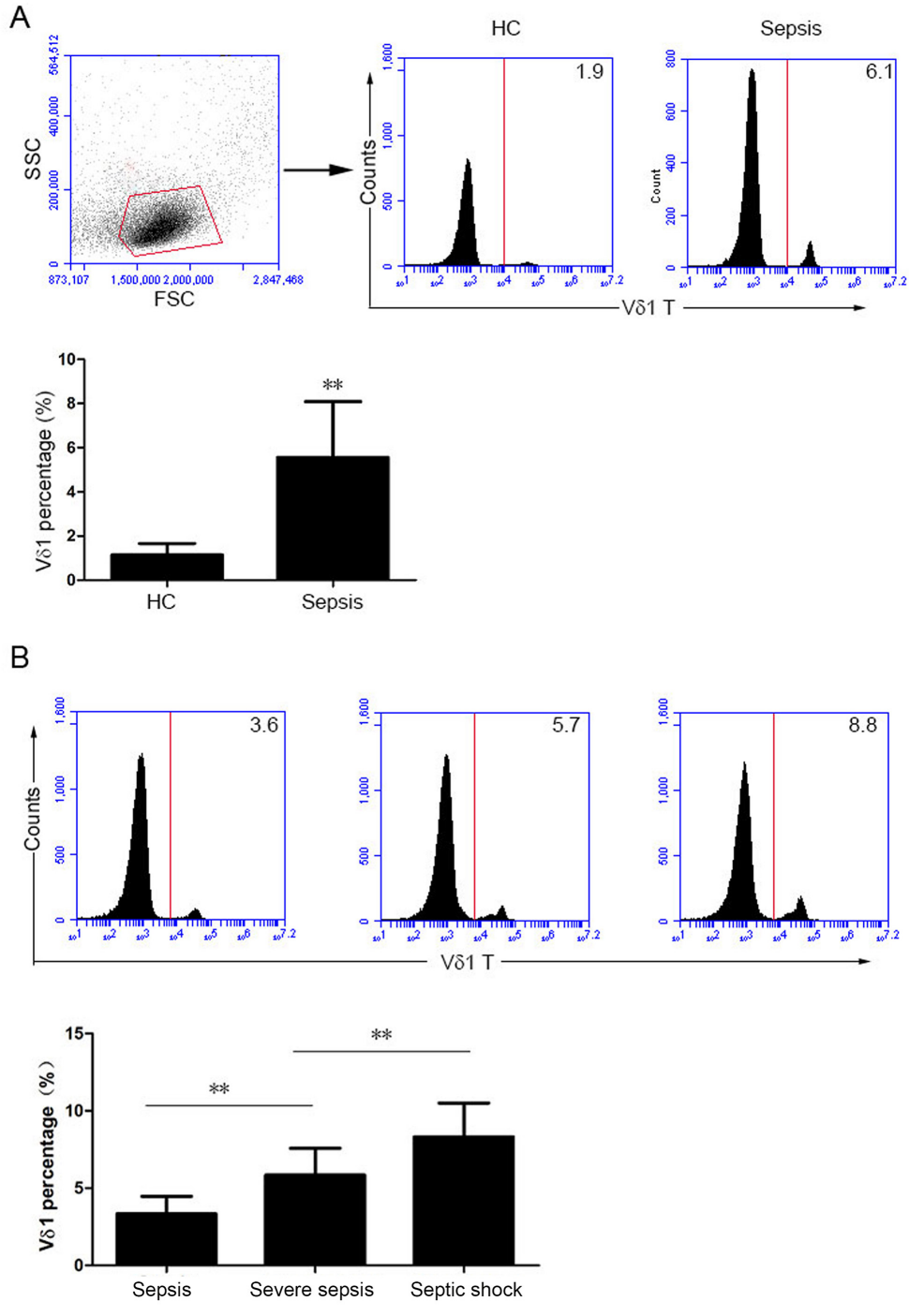


**Figure 1.** Detection of the proportion of Vδ1T cells in the peripheral blood. Flow cytometry was used to detect the proportion of Vδ1T cells in peripheral blood of the healthy controls and septic patients (A), and the ratio of Vδ1T cells in peripheral blood of the patients with sepsis in different conditions (B). **P<0.01 *vs*. the health control.

Vδ1T cells in the peripheral blood of the healthy controls (HC) and septic patients was 1.15±0.51%, and 5.56±2.53%, respectively, and the proportion of Vδ1T cells in the peripheral blood of patients with sepsis was significantly higher than that of the HC (P<0.01; Figure 1A). The proportion of Vδ1T cells in the peripheral blood of septic patients was observed in different conditions. The results in Figure 1B showed that the proportion of Vδ1T cells was 3.34±1.13% in the peripheral blood of patients with moderate sepsis, 5.84±1.74% in patients with severe sepsis, and 8.32±2.18% in patients with septic

shock. With the exacerbation of sepsis, the proportion of Vδ1T cells in the peripheral blood gradually increased (P<0.01).

*Correlation between the Ratio of Vδ1T Cells in the Peripheral Blood of Septic Patients and Their Condition*

Correlation between the ratio of Vδ1T cells in the peripheral blood of septic patients and their condition was observed and the results are shown in Figure 2. The proportion of Vδ1T cells in septic patients was positively correlated with the SOFA score (r=0.4204, P<0.01; Figure 2A), APACHE II score (r=0.4744, P<0.01; Figure 2B), and lactic acid level (r=0.3570, P<0.05; Figure 2C). The proportion of Vδ1T cells in survival patients with sepsis was lower than in non-survival septic patients (P<0.01; Figure 2D).

*Treatment Changed the Proportion of Vδ1T Cells in the Peripheral Blood of Septic Patients*

The proportion of Vδ1T cells in the blood of septic patients was compared before and after treatment. Compared with before treatment, the proportion of Vδ1T cells in the peripheral blood of septic patients after treatment reduced (5.56%±2.53% *vs* 2.98±1.43%) (P<0.01; Figure 3B).

*Expression of Immunosuppressive Molecules on Vδ1T Cells in the Peripheral Blood in Septic Patients*

The expression of immunosuppressive molecules on Vδ1T cells in the peripheral blood in patients with sepsis and the healthy controls was measured. The percentage of GITR-, CTLA-4-, and TIM-3- positive Vδ1T cells in the peripheral blood of the healthy controls was 7.34±3.52%, 3.82±1.62%, and 12.8±3.31%, respectively. The percentage of GITR-, CTLA-4-, and TIM-3- positive Vδ1T cells in the peripheral blood of the septic patients was 30.2±6.36%, 13.1±4.27%, and 28.1±6.36%%, respectively. Compared with the healthy controls, the percentage of GITR-, CTLA-4-, and TIM-3- positive Vδ1T cells in the peripheral blood of septic patients significantly increased (P<0.01; Figure 4).

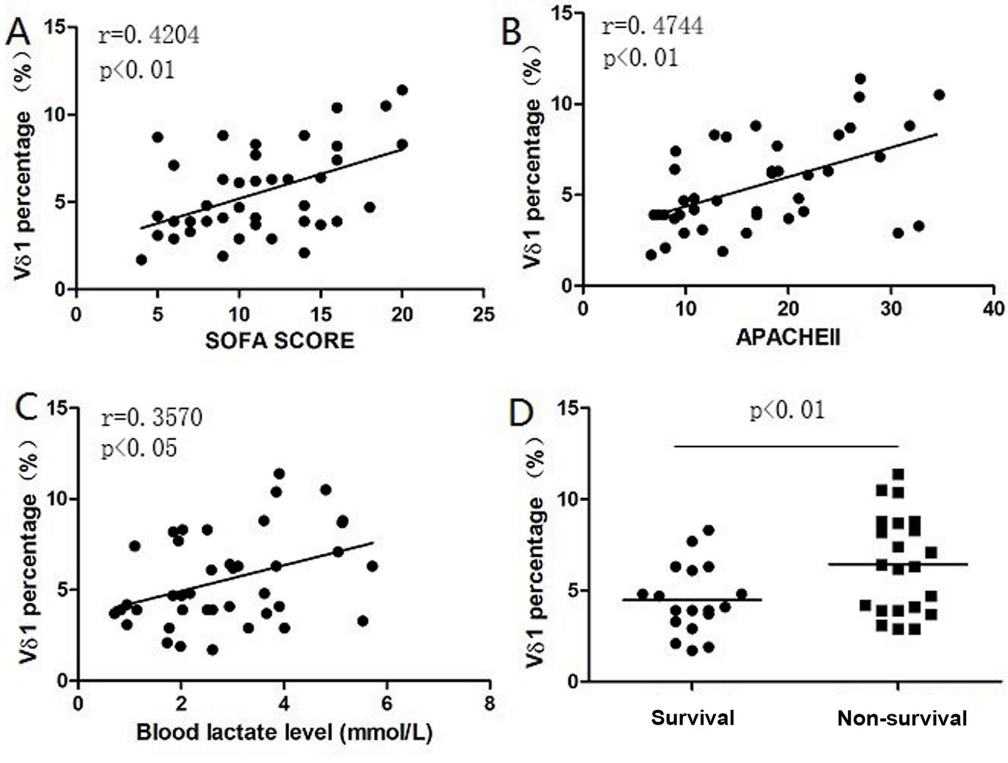


**Figure 2.** The proportion of Vδ1T cells in septic patients positively correlated with their condition. Correlation between Vδ1T cells in the peripheral blood is the SOFA score (A), APACHE II score (B), the lactate level (C), and the patient survival rate (D). $^{**}$P<0.01 *vs*. the survival group.

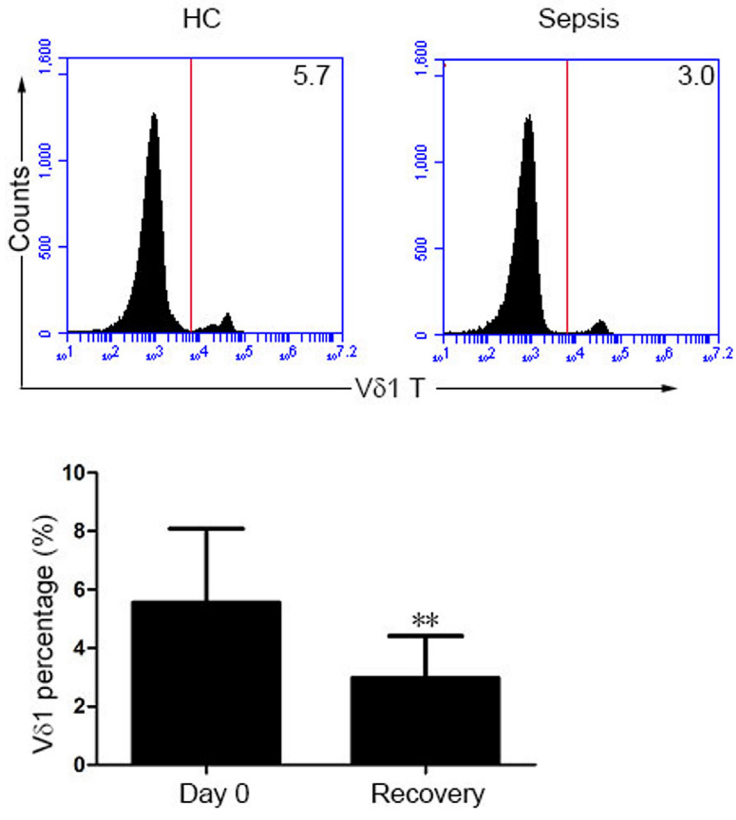


**Figure 3.** Proportion of Vδ1T cells in the peripheral blood of patients with sepsis after treatment. Flow cytometry was used to detect the proportion of Vδ1T cells in peripheral blood of septic patients before and after treatment (A). The proportion of Vδ1T cells significantly decreased after treatment (B). **P<0.01 *vs.* the Day 0 group.

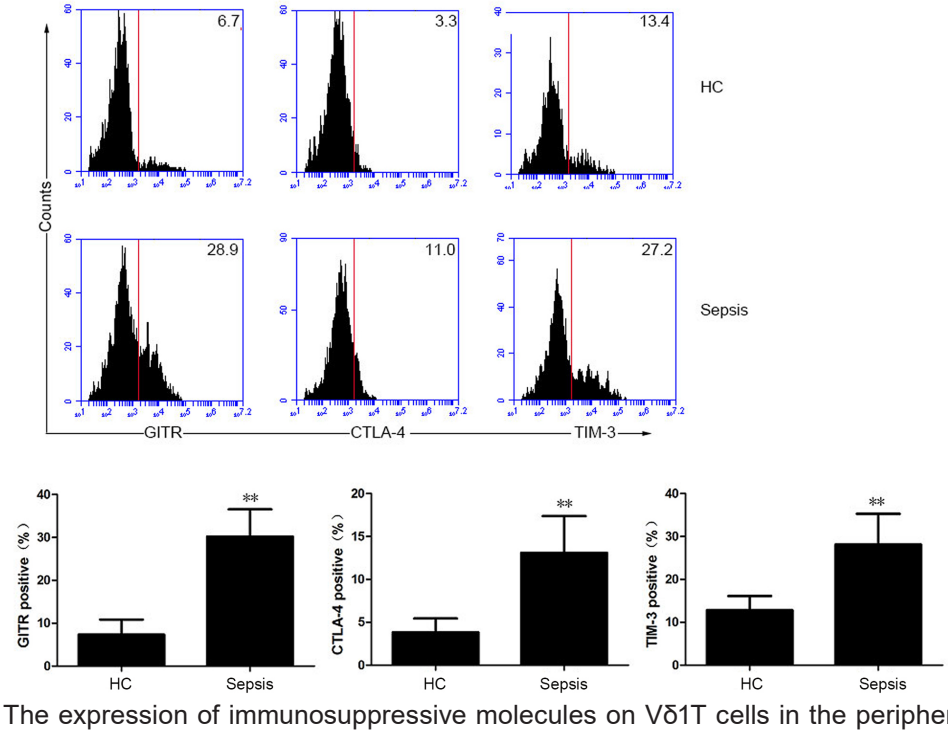


**Figure 4.** The expression of immunosuppressive molecules on Vδ1T cells in the peripheral blood of patients with sepsis. Flow cytometry was used to detect the expression of GITR, CTLA-4, and TIM-3 on Vδ1T cells in sepsis patients increased (Figure 4A-B). **P<0.01 *vs.* the Health control group. GITR, glucocorticoid-induced tumor necrosis factor receptor; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; TIM-3, T cell immunoglobulin and mucin domain 3.

*Vδ1T Cells Inhibited the Proliferation of and IFN-γ Secretion by CD4 T Cells*

The proliferation level of CD4 T cells in the peripheral blood of HC was 95.2±6.83%, and it reduced to 70.7±7.92% after the Vδ1T and CD4 T cells were incubated. The proliferation level of CD4 T cells in the peripheral blood of patients with sepsis was 99.5±8.14%, and it was changed to 51.1±8.01% after the Vδ1T cells were added (Figure 5A). It indicated that the increase of Vδ1T cells in the sepsis patients had inhibitory effects on T cell proliferation. The IFN-γ secreted by CD4 T cells in the peripheral blood of the HC was 34.1±5.66%, and it was 20.4±6.21% after the Vδ1T and CD4 T cells were incubated. The IFN-γ secreted by CD4 T cells in the peripheral blood of septic patients was 35.4±8.82%, but it changed to 15.8±4.95% in co-incubated Vδ1T and CD4 T cells from septic patients (Figure 5B). These results suggest that the immunosuppressive function of Vδ1T cells in the peripheral blood of septic patients is significantly higher than that of the healthy controls (P<0.01; Figure 5).

## DISCUSSION

γδT cells represent a minor population of T lymphocytes in human peripheral blood, which can directly recognize and bind antigens and play a crucial role in immune surveillance and regulation (31). In the healthy controls, Vδ1T cells are found predominately

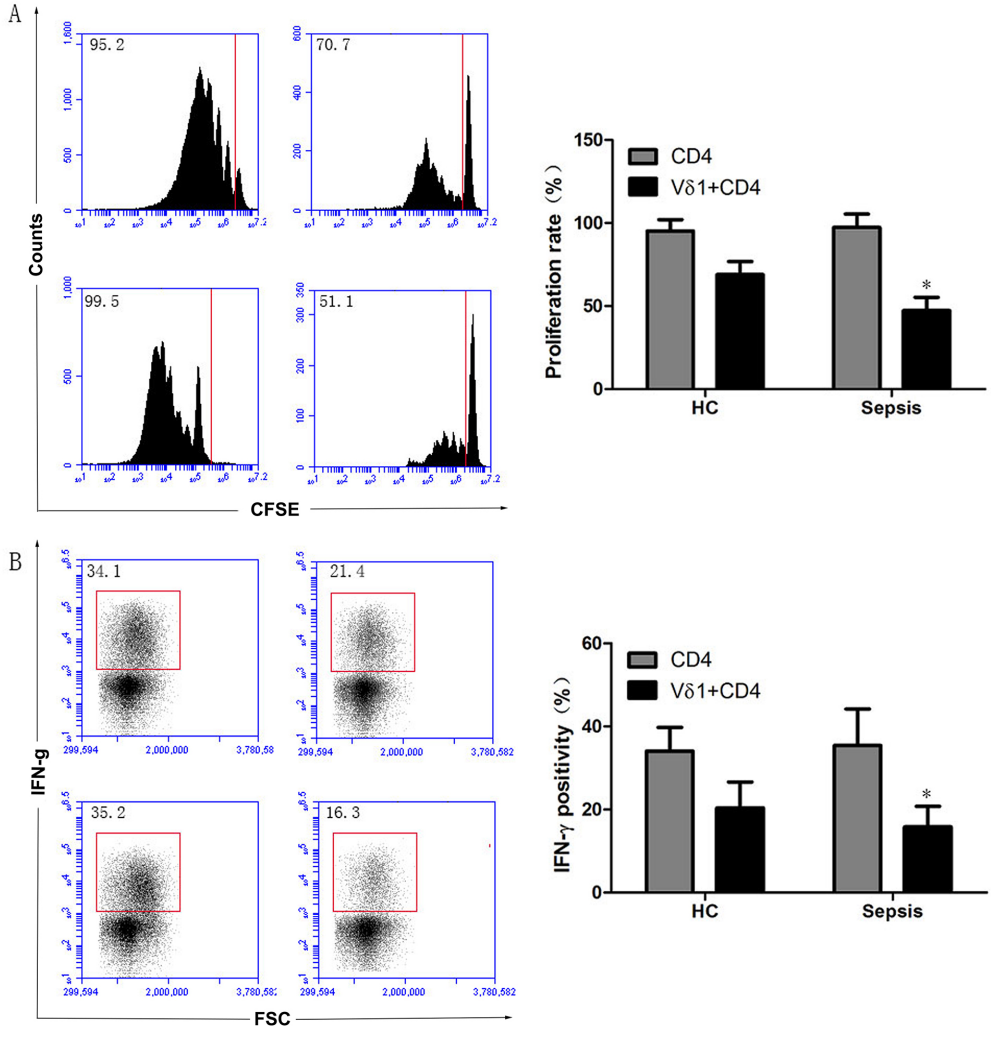


**Figure 5.** Vδ1T cells inhibited the proliferation of CD4 T cells. Flow cytometry showed that CD4 T and Vδ1T cells were incubated in peripheral blood of septic patients, CD4 T cell proliferation (A) and IFN-γ secretion ability (B) significantly decreased compared with CD4T cell culture alone. **P<0.01 vs. the healthy control group. IFN-γ, interferon-γ. The histograms are just representative examples of data.

at mucosal sites and can respond to non-classical MHC molecules expressed on stressed cells (32). Conversely, Vδ2T cells represent among 70%of circulating γδT cell subsets, and respond to phosphor antigens without MHC restriction (33). In this study, we found that the proportion of Vδ1T cells in the blood of septic patients is higher than that of HC, which positively correlated with the severity of the disease. After some effective treatment, the percentage of Vδ1T cells in the peripheral blood of patients with sepsis significantly reduced. In addition, the percentage of GITR-, CTLA-4-C, and TIM-3- positive Vδ1T cells in the blood of septic patients is significantly higher than that of the healthy controls, and the immunosuppressive function of Vδ1T cells in the peripheral blood of septic patients is significantly higher than that of the controls.

A large amount of experimental and clinical studies have shown that sepsis can cause remission of immunosuppression in the body, which in turn leads to priming infections, mainly opportunistic infections (28). Consistent with this evidence, the proportion of peripheral blood regulatory T (Treg) cells in the peripheral blood of patients with septic shock significantly increased (34). Wan et al. (2010) showed that during the early stages of sepsis, a significant proportion of abnormal Treg cells exists which is mainly manifested by an increase in the proportion and enhancement of immunosuppression (13). In addition, many studies have confirmed the abnormal proportion and function of Treg in septic patients. For instance, Huang et al. (2015) have found a significant increase in the proportion of $CD39^+$Treg in the peripheral blood of septic patients, and the increase in the proportion of $CD39^+$Treg in the peripheral blood of septic patients is closely related to the prognosis of patients. Shao et al. (2011) provided evidence that the proportion of $CD4^+CD25^+$Treg cells in the peripheral blood of septic patients significantly increased (14). The increase in the proportion of $CD4^+CD25^+$Treg cells can lead to a decrease in $CD3^+$, $CD4^+$, and $CD4^+/CD8^+$ levels, which can inhibit immune function and participate in the pathogenesis of sepsis (15). In addition, the study by Pagel et al. (2016) confirmed that the proportion of $CD4^+CD25^+$Treg cells significantly increased in patients with clinical premature sepsis in preterm infants (16). In recent years, Vδ1T cells have been proven to play an immunosuppressive function in autoimmune diseases and tumor escape (23-26). Referring to the previous results, the role of Treg cells in the pathogenesis of sepsis, and the state of immunosuppression in patients with sepsis, we suspect that Vδ1T cells are involved in the development of sepsis and are associated with patient prognosis. Our results confirmed that the proportion of Vδ1T cells in the peripheral blood increased in septic patients, and shows a positive correlation with the severity of the disease. At the same time, the proportion of Vδ1T cells in septic patients was closely related to the patients' condition. These results are consistent with the changes observed in the immunosuppressive cells in most studies involving patients with sepsis. Based on the above-mentioned findings, we can preliminarily conclude that Vδ1T cells are closely related to the development and prognosis of sepsis. According to the conclusion of the comprehensive literature, the incidence of sepsis is not a result of a change in the proportion or function of immunosuppressive cells but related to a variety of immunosuppressive cells including Treg and Vδ1T cells, which are jointly related to the development of sepsis. Future regulatory DC cells and regulatory B cells that are not much concerned in sepsis patients will also be the focus of research in sepsis mechanisms.

Some studies have shown that in patients with tumors, the function of Vδ1T cells, in addition to the changes in proportion, mainly includes enhanced proliferation capacity and enhanced immune suppressive functions (35-37). The immunosuppressive state of septic patients is similar to that of those who have

tumors, and the changes in the function of Vδ1T cells in septic patients are also being researched. A recent study evaluated the characterization of Vδ1 and Vδ2 T cells in the peripheral blood of septic patients, indicating the involvement of imbalance and functional changes of Vδ1 and Vδ2 T in sepsis progression (38). This study indicated that the percentage of GITR-, CTLA-4-, and TIM-3-positive Vδ1T cells in the peripheral blood of septic patients is significantly higher than that of HC, and the inhibition of naïve CD4 T-cell proliferation and ability of Vδ1T cells in the peripheral blood to secrete cytokines in septic patients are significantly higher than that of the controls.

## CONCLUSION

In summary, our study suggested that the proportion of Vδ1T cells in the blood of patients with sepsis significantly increased, and their function significantly enhanced. The immune function of septic patients is inhibited. Changes in the immune function of Vδ1T cells in septic patients may be closely related to the preconditioning of patients with sepsis.

## ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study is approved by the Ethics Committee of Huangshi Central Hospital of Edong Healthcare Group. Written informed consent was obtained.

## AUTHOR'S CONTRIBUTION

Fawei Yuan carried out study concepts and design, experimental studies and data acquisition; Huan Yin helped to manuscript preparation and editing; Juan Tan contributed to literature research and data analysis; Kun Zheng was the guarantor of integrity of the entire study and helped to clinical studies; Xiping Mei carried out definition of intellectual content and statistical analysis; Lixue Yuan helped to manuscript review.

**Conflict of Interest:** None declared.

## REFERENCES


1. Nam M, Son BH, Seo JE. Improved Diagnostic and Prognostic Power of Combined Delta Neutrophil Index and Mean Platelet Volume in Pediatric Sepsis. Ann ClinLab Sci. 2018; 48(2):223-230.
2. Garcia-Alvarez M, Marik P, Bellomo R. Sepsis-associated hyperlactatemia. Crit Care 2014; 18(5):503.
3. Balk RA. Severe sepsis and septic shock. Definitions, epidemiology, and clinical manifestations. Crit Care Clin. 2000; 16(2):179-192.
4. Angus DC, Wax RS. Epidemiology of sepsis: an update[J]. Crit Care Med 2001; 29(7 Suppl):S109-116.
5. Molnár L, Fülesdi B, Németh N. Sepsis-associated encephalopathy: A review of literature. Neurol India. 2018; 66(2):352-361.
6. Bone RC. The sepsis syndrome. Definition and general approach to management[J]. Clin Chest Med. 1996; 17(2):175-181.
7. Patil NK, Bohannon JK, Sherwood ER. Immunotherapy: A promising approach to reverse sepsis-induced immunosuppression. Pharmacol Res. 2016; 111:688-702.
8. Boomer JS, To K, Chang KC. Immunosuppression in patients who die of sepsis and multiple organ failure. JAMA. 2011; 306(23):2594-2605.
9. Lelubre C, Vincent JL. Mechanisms and treatment of organ failure in sepsis. Nat Rev Nephrol. 2018; 14(7):417-427.
10. Patil NK, Guo Y, Luan L. Targeting Immune Cell Checkpoints during Sepsis. Int J Mol Sci. 2017; 18(11):2413.
11. Hotchkiss RS, Monneret G, Payen D. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. Nat Rev Immunol. 2013; 13(12):862-874.
12. Hiraki S, Ono S, Tsujimoto H. Neutralization of interleukin-10 or transforming growth factor-β

decreases the percentages of CD4+ CD25+ Foxp3+ regulatory T cells in septic mice, thereby leading to an improved survival. Surgery. 2012; 151(2):313-322.
13. Wan YY. Regulatory T cells: immune suppression and beyond. Cell Mol Immunol. 2010; 7(3):204-210.
14. Huang H, Xu R, Lin F, Bao C, Wang S, Ji C, et al. High circulating CD39(+) regulatory T cells predict poor survival for sepsis patients. Int J Infect Dis, 2015; 30:57-63.
15. Shao M, Liu B, Wang JQ, Tao XG, Zhou SS, Jin K, et al. Clinical significance of CD4+CD25+ T cell examination in sepsis patients. J Hunan Chin Med U. 2011; 31(4):8-10.
16. Pagel J, Hartz A, Figge J, Gille C, Eschweiler S, Petersen K, et al. Regulatory T cell frequencies are increased in preterm infants with clinical early-onset sepsis. Clin Exp Immunol. 2016; 185(2):219-227.
17. Haas W, Pereira P, Tonegawa S. Gamma/delta cells. Annu Rev Immunol. 1993; 11:637-685.
18. Kabelitz D, Wesch D, He W. Perspectives of gammadelta T cells in tumor immunology. Cancer Res. 2007; 67:5-8.
19. Hayday AC. γδ cells: A right time and a right place for a conserved third way of protection. Annu Rev Immunol. 2000; 18:975-1026.
20. Harly C, Peyrat MA, Netzer S, et al. Up-regulation of cytolytic functions of human Vdelta2-gamma T lymphocytes through engagement of ILT2 expressed by tumor target cells. Blood. 2011; 117:2864-2873.
21. Kabelitz D, Kalyan S, Oberg HH, Wesch D. Human Vδ2 versus non-Vδ2 γδ T cells in antitumor immunity. Oncoimmunology. 2013; 2:e23304.
22. Zhao H, Xi X, Cui L, He W. CDR3δ-grafted γ9δ2T cells mediate effective antitumor reactivity. Cell Mol Immunol. 2012; 9:147-154.
23. Kress E, Hedges JF, Jutila MA. Distinct gene expression in human Vdelta1 and Vdelta2 gammadelta T cells following non-TCR agonist stimulation. Mol Immunol. 2006;43:2002-2011.
24. Bonneville M, O'Brien RL, Born WK. Gammadelta T cell effector functions: A blend of innate programming and acquired plasticity. Nat Rev Immunol. 2010;10:467-478.
25. Peng G, Wang HY, Peng W, Kiniwa Y, Seo KH, Wang RF. Tumor-infiltrating gammadelta T cells suppress T and dendritic cell function via mechanisms controlled by a unique toll-like receptor signaling pathway. Immunity. 2007; 27(2):334-348.
26. Li X, Kang N, Zhang X, Dong X, Wei W, Cui L, et al. Generation of human regulatory gammadelta T cells by TCRgammadelta stimulation in the presence of TGF-beta and their involvement in the pathogenesis of systemic lupus erythematosus. J Immunol. 2011; 186(12):6693-6700.
27. Vavassori S, Galson JD, Trück J. Lymphadenopathy driven by TCR-Vγ8Vδ1 T-cell expansion in FAS-related autoimmune lymphoproliferative syndrome. Blood Adv. 2017; 1(15):1101-1106.
28. Papali A, Eoin West T, Verceles AC, Augustin ME, Nathalie Colas L, Jean-Francois CH, et al. Treatment outcomes after implementation of an adapted WHO protocol for severe sepsis and septic shock in Haiti. J Crit Care. 2017; 41:222-228.
29. Yue C, Yang K, Dong W, Hu F, Zhao S, Liu S. gammadelta T Cells in Peripheral Blood of Glioma Patients. Med Sci Monit. 2018; 24:1784-1792.
30. Yin S, Zhang J, Mao Y, Hu Y, Cui L, Kang N, He W. Vav1-phospholipase C-γ1 (Vav1-PLC-γ1) pathway initiated by T cell antigen receptor (TCRγδ) activation is required to overcome inhibition by ubiquitin ligase Cbl-b during γδT cell cytotoxicity. J Biol Chem. 2013; 288(37):26448-26462.
31. Willcox CR, Pitard V, Netzer S, Couzi L, Salim M, Silberzahn T, Moreau JF, Hayday AC, Willcox BE, Déchanet-Merville J. Cytomegalovirus and tumor stress surveillance by binding of a human gammadelta T cell antigen receptor to endothelial protein C receptor. Nat Immunol. 2012; 13: 872-879.
32. Groh V, Steinle A, Bauer S, Spies T. Recognition of stress-induced MHC molecules by intestinal epithelial gammadelta T cells. Science. 1998; 279:1737-1740.
33. Poccia F, Wallace M, Colizzi V, Malkovsky M. Possible protective and pathogenic roles of gamma delta T lymphocytes in HIV-infections (Review). Int J Mol Med. 1998; 1:409-413.
34. Nascimento DC, Melo PH, Piñeros AR. IL-33 contributes to sepsis-induced long-term immunosuppression by expanding the regulatory T cell population. Nat Commun. 2017; 8:14919.
35. Skrupky LP, Kerby PW, Hotchkiss RS. Advances in the management of sepsis and the understanding of key immunologic defects. Anesthesiology. 2011; 115(6):1349-1362.
36. Meraviglia S, Lo Presti E, Tosolini M. Distinctive features of tumor-infiltrating γδ T lymphocytes in human colorectal cancer. Oncoimmunology. 2017; 6(10):e1347742.
37. Rong L, Li K, Li R, Liu HM, Sun R, Liu XY. Analysis of tumor-infiltrating gamma delta T cells in rectal cancer. World J Gastroenterol. 2016; 22(13):3573-3580.
38. Wang XH, Li WJ, Zhu D, Zhao H, Chen P, Chen X. Characterization of human peripheral blood γδT cells in patients with sepsis. Exp Ther Med. 2020; 19(6):3698-3706.